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Chemical Composition and Bioactivity of a Carvacrol-Rich *Thymus algeriensis* Essential Oil from Gaser Alhaj, Libya

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Abstract:
The genus *Thymus* is well-known for its aromatic and medicinal properties. Among its species, *Thymus algeriensis* is widely distributed in the Mediterranean basin, including Libya, where environmental conditions can influence essential oil composition and bioactivity. This study aimed to characterize the chemical profile of *T. algeriensis* essential oil collected from Gaser Alhaj and evaluate its antibacterial and allelopathic activities. Essential oil was extracted by hydrodistillation and analyzed using GC-MS, revealing six major compounds, with carvacrol as the dominant constituent (77.01%), followed by γ-terpinene, thymol, *o*-cymene, *p*-cymene, and caryophyllene. Oxygenated monoterpenes constituted the majority of the oil. The oil exhibited strong antibacterial activity against *Escherichia coli* and *Salmonella gallinarum*, and demonstrated dose-dependent allelopathic effects on *Lactuca sativa* seed germination. Compared to previous studies from Libya, this Gaser Alhaj oil represents a distinct carvacrol-rich chemotype with superior biological activity, highlighting regional chemotypic variation. These findings suggest that *T. algeriensis* essential oil has promising potential as a natural antimicrobial agent and eco-friendly bioherbicide.

Keywords: *Thymus Algeriensis* Essential Oil, Antibacterial Activity, Allelopathy.

التركيب الكيميائي والنشاط الحيوي لزيت الزعتر الجزائري (*Thymus algeriensis*) الغني بالكارفاكرول من قصر الحاج، ليبيا

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الملخص:
يُعرف جنس الزعتر (Thymus) بخصائصه العطرية والطبية. ينتشر الزعتر الجزائري(*Thymus algeriensis*) ، أحد أنواع الجنس (Thymus)، على نطاق واسع في حوض البحر الأبيض المتوسط، بما في ذلك ليبيا، حيث تؤثر الظروف البيئية على تركيب الزيت العطري ونشاطه الحيوي. هدفت هذه الدراسة إلى توصيف التركيب الكيميائي للزيت العطري الزعتر الجزائري (*Thymus algeriensis*) الذي تم جمعه من قصر الحاج، وتقييم أنشطته المضادة للبكتيريا والأليلوباثية. استُخلص الزيت العطري بالتقطير المائي، باستخدام جهاز (Clevenger-type apparatus) وحُلِّل باستخدام كروماتوغرافيا الغاز-مطياف الكتلة(GC-MS) ، وكشف عن ست مركبات رئيسية، كان الكارفاكرول هو المكون السائد (77.01%)، يليه جاما-تربينين، والثيمول، والأوسيمين، والبارا-سيمين، والكاريوفيلين. شكلت التربينات الأحادية المؤكسدة النسبة الأكبر من الزيت. أظهر الزيت نشاطًا مضادًا للبكتيريا قويًا ضد الإشريكية القولونية والسالمونيلا الغالينية، وأظهر تأثيرات أليلوباثية تعتمد على الجرعة على إنبات بذور الخس. بالمقارنة مع دراسات سابقة من ليبيا، يُمثل زيت قصر الحاج هذا نمطًا كيميائيًا متميزًا غنيًا بالكارفاكرول، يتميز بنشاط بيولوجي فائق، مما يُبرز التباين الكيميائي.الإقليمي. تشير هذه

النتائج إلى أن زيت الزعتر الجزائري العطري يتمتع بإمكانيات واعدة كعامل طبيعي مضاد للميكروبات ومبيد أعشاب حيوي صديق للبيئة.

الكلمات المفتاحية: زيت الزعتر الجزائري العطري، النشاط المضاد للبكتيريا، النشاط الأليلوباثي.

Introduction

Increasing global need for natural products has accelerated research into medicinal herbs as viable sources of effective and safe alternatives to man-made chemicals [1]. Of these, the Thymus genus (Lamiaceae) is highly valued, and Thymus algeriensis Boiss. & Reut. is among the major species in traditional medicine in North Africa, used to treat symptoms ranging from respiratory infections to inflammatory diseases [2]. The efficacy of the plant is attributed to its rich and varied phytochemistry, i.e., its essential oil that is full of bioactive moieties in the form of thymol, carvacrol, and 1,8-cineole, whose composition varies extensively based on climatic and geographic factors [3],[4].

Early studies of Libyan Thymus species indicate the presence of efficient, geographically-specific chemotypes. For instance, Thymus capitatus from Zintan had a carvacrol-rich oil with high antimicrobial and allelopathic activity [5],[6]. On the other hand, the chemical profile and related biological activity of T. algeriensis from some Libyan regions like Gaser Alhaj remain poorly studied and may bear a distinctive chemotype due to the arid local environment.

Accordingly, this study aims to determine the chemical composition of T. algeriensis from Gaser Alhaj, Libya, and to determine its antimicrobial and phytotoxic (allelopathic) activity. The results will be a comparative perspective with the established bioactivity of Zintan T. capitatus, giving value to Libyan flora and establishing its potential for the production of natural biocontrol agents.

Materials and Methods

Plant Collection and Essential Oil Extraction

Aerial parts of Thymus algeriensis Boiss. were collected during the flowering stage in April 2021 from Gaser Alhaj, Libya. The plant was taxonomically identified by Dr. A. Felaly (Faculty of Agriculture, University of Zintan, Libya). A voucher specimen was preserved for further use. The plant material was shade-dried at room temperature for 10 days [7].

Isolation of the essential oil was conducted using hydrodistillation using a Clevenger-type apparatus in accordance with the standard method [8]. Briefly, 100 g of dried air parts were subjected to hydrodistillation for 2 h. Hydrodistilled oil was dried with anhydrous sodium sulfate and stored in amber vials at 4°C for future use [7],[9].

Chemical Characterization of the Essential Oil

The chemical composition of the essential oil was analyzed by Gas Chromatography–Mass Spectrometry (GC–MS) following the standard protocol [10]. The analysis was performed on an HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness). Helium gas was used as the carrier at 1 mL/min. The oven temperature protocol was: 60°C initial temperature (held at 2 min), subsequently ramped up to 220°C at 3°C/min and held at 10 min. The injector and detector temperatures were maintained at 250°C and 300°C, respectively.

Identification of the compounds was achieved via comparison of their mass spectra with spectra from NIST and Wiley libraries as well as through the calculation of retention indices (RI) versus a homologous series of n-alkanes (C_8–C_{22}) [10],[9].

Allelopathic Activity Assay

Allelopathic activity of the essential oil was evaluated on Lactuca sativa L. (lettuce) seed germination using a modified protocol from an earlier study [11]. The seeds were surface sterilized with 70% ethanol for 15 s and subsequently three times with distilled water.

Three dozen seeds were placed in sterile Petri dishes (90 mm diameter) with two layers of Whatman No. 1 filter paper. All the samples were subjected to 3 mL of distilled water (control) or water solutions of the essential oil at concentrations of 0, 1, 2, 3 and 4 µL/mL. The oil was first dissolved in ethanol (1:1 v/v) and diluted with distilled water to prepare stock solution (10 µL/mL), which was later diluted to working concentrations. Ethanol-water mixture control treatments showed no phytotoxic effect.

Petri dishes were incubated at 20 ± 1°C. Germination was monitored daily, and one seed was considered to be germinated if the radicle was ≥1 mm. Rotating water was added from time to time as needed to supply moisture. Three replicates for every treatment were performed.

Germination percentage (GP) and inhibition percentage (IP) were calculated using the following formulas [11]:

$$GP\ (\%) = (n / N) \times 100$$
$$IP\ (\%) = [(C_n - T_n) / C_n] \times 100.$$

where:

n = seeds germinated in the treatment, N = total number of seeds,
C_n = number of germinated seeds in the control, T_n = number of seeds germinated in the treatment.

Test for Antibacterial Activity
Antibacterial activity of the essential oil was determined against *Escherichia coli* and *Salmonella gallinarum* using the standard disk diffusion assay [7],[12]. The essential oil was diluted in dimethyl sulfoxide (DMSO) to a concentration of 5% (v/v) before application to the disks. Bacterial suspensions were standardized at 10^5 CFU/mL and swabbed on Mueller-Hinton agar plates. Sterile filter paper disks of 6 mm in diameter were loaded with 10 µL of the diluted essential oil and positioned on the inoculated agar. Gentamicin (15 µg/disk) was used as a positive control, whereas disks loaded with DMSO only (5% v/v) were used as negative controls.
Plates were incubated at 37°C for 24 hours. Inhibition zone diameters (including the disk diameter) were measured in millimeters. All experiments were performed in triplicate.

Results
Chemical Composition of the Essential Oil
Water hydrodistillation of the aerial parts of *Thymus algeriensis* yielded an essential oil which was subsequently analyzed by GC-MS. The analysis revealed a total of 22 identified compounds, representing 97.79% of the total oil composition (Table 1). The dominant compound was carvacrol, representing 77.01% of the total oil. Other significant constituents included γ-terpinene (5.44%), thymol (4.11%), *o*-cymene (3.90%), *p*-cymene (2.63%), and caryophyllene (1.24%).
Classification of the identified compounds by chemical class showed that oxygenated monoterpenes were the most abundant group, accounting for 82.94% of the total oil composition. Monoterpene hydrocarbons represented 13.38%, sesquiterpenes 1.48%, and aromatic compounds 6.53%. The total identified composition was 97.79%.

Table 1. Chemical composition of the essential oil from *Thymus algeriensis* collected in Gaser Alhaj

Compound	RIa	%
α-thujene	923	0.12
α-pinene	929	0.40
Camphene	941	0.07
β-pinene	968	0.02
Myrcene	984	0.35
α-phellandrene	1002	0.08
Δ (3) Carene	1007	0.01
α-terpinene	1012	0.32
o-cymene	1018	3.90
p-cymene	1019	2.63
γ-terpinene	1050	5.44
Terpinolene	1079	0.04
Linalool	1090	0.51
Borneol	1161	0.26
Terpinen-4-ol	1173	0.21
α-terpineol	1191	0.24
Thymol	1290	4.11
Carvacrol	1303	77.01
Caryophyllene	1414	1.24
α-humulene	1450	0.03
Caryophyllene oxide	1579	0.21
1,8-cineole (Eucalyptol)	1033	0.40
Estragole (Methyl chavicol)	1195	0.20
Total		**97.79**
Class		Percentage
A: Monoterpenes		13.38%
B: Oxygenated Monoterpenes		82.94%
C: Sesquiterpenes		1.48%
D: Aromatic Compounds		6.53%
Total		97.79%

Antibacterial Activity

The essential oil exhibited potent in vitro antibacterial activity against the tested Gram-negative pathogens (Table 2). The inhibition zone diameters for the *T. algeriensis* essential oil were **38 mm** against *Escherichia coli* and **36 mm** against *Salmonella gallinarum*. Notably, the antibacterial activity of the essential oil surpassed that of the standard antibiotic gentamicin (15 µg), which produced zones of inhibition of 14 mm and 16 mm for *E. coli* and *S. gallinarum*, respectively.

Table 2. Inhibition zones (mm) of essential oil and gentamycin.

Species	Essential oil	Gentamycin (15 µg)
E. coli	38	14
S. gallinarum	36	16

Allelopathic Activity

The allelopathic effective of the essential oil was assessed through a germination assay using *Lactuca sativa* L. seeds at four different concentrations (1, 2, 3, and 4 µl/ml) are summarized in (Figure.1). The results showed a clear concentration-dependent inhibitory effect on seed germination. The inhibition percentage (IP) increased from 6.67% to 43.33%, while the germination percentage (GP) correspondingly decreased from 93.33% to 56.67% across the tested concentration range. This is in stark contrast to the control treatment (0 µl/ml), which exhibited an IP of 0% and a GP of 100%.

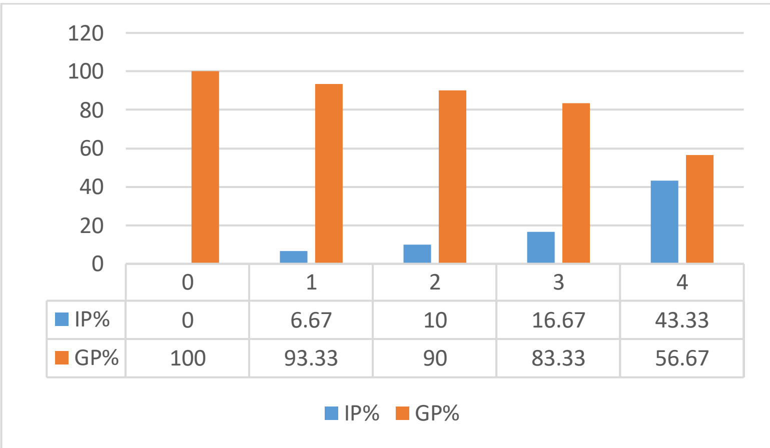

Figure 1. Dose-dependent allelopathic effect of *Thymus algeriensis* essential oil on *Lactuca sativa*. Seeds

Discussion

Chemical Characterization of the Essential Oil

The present study demonstrates that the essential oil of *Thymus algeriensis* from Gaser Alhaj, Libya, is a rich source of bioactive compounds, primarily phenolic monoterpenes, responsible for its significant antibacterial and allelopathic properties. The chemical profile of the analyzed *T. algeriensis* oil, dominated by carvacrol (77.01%), together with other notable constituents such as thymol (4.11%), γ-terpinene (5.44%), o-cymene (3.90%), p-cymene (2.63%), and caryophyllene (1.24%), classifies it as a carvacrol chemotype. This finding aligns with previous studies on North African *Thymus* species but also highlights the remarkable chemical polymorphism within *T. algeriensis* [4],[7]. For instance, Giweli et al. [4] reported a thymol chemotype (38.5%) from Zintan, Libya, whereas studies from Algeria and Morocco have reported chemotypes rich in camphor, 1,8-cineole, α-pinene, or terpinyl acetate [7],[13],[3],[14]. Such chemical variability is often attributed to geographic origin, local climate, soil conditions, and harvest time [7],[13],[3].

The class distribution in the present oil further supports its bioactivity profile. Oxygenated monoterpenes were the predominant class (82.94%), followed by monoterpenes (13.38%), aromatic compounds (6.53%), and sesquiterpenes (1.48%). This predominance of oxygenated monoterpenes, particularly carvacrol and thymol, is noteworthy since they are frequently associated with strong antibacterial, antioxidant, and allelopathic effects

[4],[5]. The relatively lower proportions of monoterpenes and sesquiterpenes may also contribute synergistically to the observed biological activities.

Antibacterial Activity

The antibacterial activity observed against both Gram-negative (*E. coli, S. gallinarum*) is directly linked to the high phenolic content of the oil. Phenolic compounds such as carvacrol (77.01%) and thymol (4.11%) are known to disrupt microbial cell membranes, increasing permeability and causing leakage of cellular contents [4],[5]. In our oil, oxygenated monoterpenes constituted the major class (82.94%), largely dominated by carvacrol and thymol, which explains the potent antibacterial action. The presence of minor oxygenated constituents such as linalool and borneol, even in low concentrations, may enhance this effect through synergistic interactions [4],[7]. Our findings, showed inhibition zones more than those of gentamycin, are consistent with previous reports. For example, *Thymus capitatus* oil (68.19% carvacrol) from Libya showed superior antibacterial activity compared to gentamycin [5]. Similarly, *T. algeriensis* oils from other North African regions demonstrated strong efficacy against a range of foodborne pathogens [4],[7]. The slightly higher sensitivity of *S. gallinarum* compared to *E. coli* may reflect structural differences in cell walls; the outer membrane of Gram-negative bacteria can hinder hydrophobic compounds, though potent phenols like carvacrol remain effective [7],[15],[16]. The contribution of monoterpenes such as γ-terpinene (5.44%) and p-cymene (2.63%) is also notable, since these hydrocarbons can facilitate penetration of phenols into bacterial membranes, thereby reinforcing the observed activity [4],[5].

Allelopathic Activity

The strong allelopathic effects on *Lactuca sativa* seed germination, displaying a clear dose-dependent response with inhibition reaching 43.33% at 4 µL/mL, further highlight the significant bioactivity of this carvacrol-rich essential oil. The predominance of oxygenated monoterpenes (82.94%), particularly carvacrol and thymol, is strongly associated with phytotoxic properties [5],[17]. These compounds are believed to interfere with enzymatic processes in germinating seeds, such as amylase activity, and may induce oxidative stress, thereby inhibiting seedling growth [17].

The progressive suppression of germination from 6.67% to 43.33% inhibition across the tested concentrations suggests that the phytotoxic activity is not an all-or-nothing effect but a graduated response to the concentration of bioactive compounds. This pattern is consistent with studies where complete essential oils exhibit more potent activity than their major components alone, hinting at possible synergistic interactions between carvacrol and minor constituents [4],[5],[18]. The relatively lower percentages of monoterpenes (13.38%) and sesquiterpenes (1.48%), such as γ-terpinene, p-cymene, and caryophyllene, may further support this activity by modulating volatility, stability, or uptake of the major phenolics [4],[7].

Conclusion

This study reveals that the essential oil from *Thymus algeriensis* in Gaser Alhaj, Libya, is a remarkably potent, carvacrol-rich chemotype, with this single compound making up over three-quarters of its composition. The overwhelming majority of the oil consists of oxygenated monoterpenes, a class known for strong biological activity. This chemical signature stands in stark contrast to the thymol-dominated oil previously found in Zintan, highlighting how much a plant's chemistry can vary from one region to another. Accordingly, the oil showed impressive real-world effects: it was powerfully antibacterial—even more so than a standard antibiotic—and effectively suppressed seed germination in a concentration-dependent manner. Together, these robust results position this specific Libyan essential oil as a highly promising candidate for natural alternatives to synthetic antibiotics and herbicides, paving the way for its future application in medicine and sustainable agriculture."

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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