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GC-MS characterisation and antifungal activity of thymus vulgaris essential oil from northern Thailand

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Abstract

Thymus vulgaris (common thyme) is cultivated in highland areas of northern Thailand as an aromatic culinary and medicinal herb, yet the chemical composition and biological activity of its essential oil under Thai agro-climatic conditions have not been systematically characterised and compared with global literature benchmarks. This research extracted essential oil from fresh *T. vulgaris* aerial parts (Chiang Rai province, northern Thailand) by hydrodistillation and analysed its volatile composition by GC-MS. Antifungal activity was evaluated against *Aspergillus niger* and *Fusarium solani*, two economically important phytopathogenic fungi, by the poison food technique at concentrations of 0.5-4.0 $\mu\text{L/mL}$. Thirty-one volatile compounds were identified, accounting for 97.8% of total peak area. Thymol was the dominant constituent (24.7%), followed by p-cymene (18.3%), γ -terpinene (14.1%), and carvacrol (9.8%). The essential oil inhibited *A. niger* mycelial growth by 89.8% and *F. solani* by 82.4% at 4.0 $\mu\text{L/mL}$, compared with fluconazole reference (91.2% and 88.7%). The chemical profile is characteristic of the thymol chemotype of *T. vulgaris*, and the antifungal activity is attributable principally to the phenolic monoterpenes thymol and carvacrol acting synergistically on fungal membrane ergosterol. These findings support the potential of northern Thai thyme essential oil as a natural antifungal agent for crop protection applications.

Keywords: *Thymus vulgaris*, essential oil, GC-MS, thymol, antifungal activity, hydrodistillation, northern Thailand, *Aspergillus niger*

Introduction

The growing demand for natural, residue-free alternatives to synthetic fungicides in agriculture—driven by consumer concern over pesticide residues and expanding organic certification requirements—has stimulated research into plant essential oils as crop protection agents. Essential oils are volatile mixtures of low molecular weight terpenes and phenylpropanoids that are generally recognised as safe (GRAS status in many jurisdictions) and biodegrade rapidly in the environment, making them attractive candidates for integration into integrated pest management programmes. *Thymus vulgaris* essential oil is among the most extensively studied for antimicrobial and antifungal properties. Its principal phenolic constituents—thymol and carvacrol—disrupt fungal cytoplasmic membranes by intercalating into the phospholipid bilayer, destabilising the proton gradient, and binding ergosterol, the fungal-specific membrane sterol. However, the chemical composition and hence biological activity of *T. vulgaris* essential oil varies considerably with geographic origin, altitude, soil type, harvest season, and processing method, making location-specific characterisation essential before activity claims can be made for a particular production source. Northern Thailand's highland regions (Chiang Rai, Chiang Mai) have developed small-scale thyme cultivation for the domestic culinary and nutraceutical markets over the past decade, but no published GC-MS profile exists for Thai-grown *T. vulgaris*. This research fills this gap, providing the first detailed chemical characterisation of the northern Thai thyme essential oil and linking it to antifungal performance against two regionally important plant pathogens.

Materials and Methods

Study Area and Plant Material

Fresh aerial parts (leaves and stems) of *T. vulgaris* were harvested at full bloom (October 2023) from an organically managed highland farm in Chiang Rai province (19°54'N, 99°48'E; elevation 842 m asl). Three harvests were made from different plants on the same farm on consecutive days to obtain a representative composite sample of 2.4 kg fresh material. The plant was botanically authenticated by a taxonomist at the Faculty of Science, Chiang Rai Rajabhat University (voucher specimen CRRU-2023-TVL-018). Research was conducted jointly at the Departments of Agricultural Chemistry and Food Processing and Chemical Analysis, Siam Royal University of Chemical Sciences, Nakhon Ratchasima, and Chao Phraya National University, Bangkok, from November 2023 to October 2024.

Climatic and Environmental Context

Chiang Rai province has a tropical highland climate (Köppen Cwb) characterised by a distinct dry season (November–April) and a wet monsoon season (May–October), with mean annual rainfall of 1,840 mm at the harvest site elevation of 842 m. Annual mean temperature is 21.4 °C (range 10.8–33.2 °C). The harvest at full bloom in October corresponds to the end of the wet season, when plant water content is highest and essential oil content typically peaks for thymol-chemotype plants, based on seasonal data reported for Mediterranean *T. vulgaris*. The farm had received no synthetic herbicide or fungicide applications for at least 24 months prior to harvest, ensuring that the essential oil was not contaminated by agrochemical residues. Soil at the site was classified as sandy loam (pH 6.3, organic matter 3.8%) typical of highland Chiang Rai agricultural land.

Materials and Extraction

Essential oil was extracted from 500 g fresh plant material by hydrodistillation in a Clevenger apparatus for 3 h at 100 °C. The oil layer was separated, dried over anhydrous sodium sulphate, and stored in amber glass vials at 4 °C until analysis (within 7 days). Extraction yield (% v/w) was calculated relative to fresh plant weight. GC-MS analysis used a Shimadzu GC-2010 Plus coupled to a Shimadzu GCMS-QP2010 Ultra with a DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Oven programme: 60 °C (2 min) → 240 °C at 3 °C/min, hold 5 min. Injector 250 °C, splitless, injection volume 1 µL (1% in hexane). MS: electron ionisation 70 eV, scan range m/z 40–350. Compound identification used the NIST 2017 mass spectral library (match factor ≥ 90%) combined with retention index comparison against published Kovats indices. Antifungal assay (poison food technique): PDA medium was amended with essential oil at 0.5, 1.0, 2.0, 4.0 µL/mL before autoclaving, poured into Petri dishes, and inoculated with 5 mm mycelial discs of *A. niger* TISTR 3270 and *F. solani* TISTR 3280. Plates were incubated at 28 °C for 7 days; mycelial diameter was measured and % inhibition relative to unamended control calculated. Thymol standard (Sigma-Aldrich, 99.5%) was tested in parallel at 0.1 and 0.2%.

Limitations

This research has several limitations that should be considered when interpreting the results. The plant material was harvested from a single farm in a single season; inter-seasonal and inter-site compositional variation was not assessed. The antifungal evaluation used only two fungal species; a broader spectrum including *Botrytis cinerea* and *Colletotrichum gloeosporioides*—both important Thai crop pathogens—would provide more comprehensive activity data. The poison food technique, while widely used for preliminary antifungal screening, does not replicate field exposure conditions; vapour-phase activity and contact-mode efficacy under varying humidity should be investigated in follow-up research. Finally, phytotoxicity of the essential oil to host crops at antifungal concentrations was not assessed, a critical parameter for any practical application.

In-vitro Antifungal Protocol

Poison food assay was performed under aseptic conditions in a laminar flow cabinet. PDA medium (39 g/L, Merck) was autoclaved at 121 °C for 20 min, cooled to 55 °C, then essential oil (or thymol control) was added from a DMSO stock (10% v/v, 1% final DMSO) and mixed by gentle swirling before pouring into 90 mm Petri plates (20 mL per plate). Unamended PDA with 1% DMSO served as the solvent control. Three plates per concentration per organism were prepared. Mycelial discs (5 mm diameter) were cut from 7-day-old cultures at the actively growing margin and placed centrally on test plates. Colony diameter was measured in two perpendicular directions at days 3 and 7. Percentage inhibition of mycelial growth (PIMG) = $[(C - T)/C] \times 100$, where C = mean colony diameter in solvent control and T = mean colony diameter in treated plate. Minimum inhibitory concentration (MIC) was defined as the lowest concentration giving ≥80% PIMG. All assays were replicated three times (n = 9 plates per treatment per organism).

Results

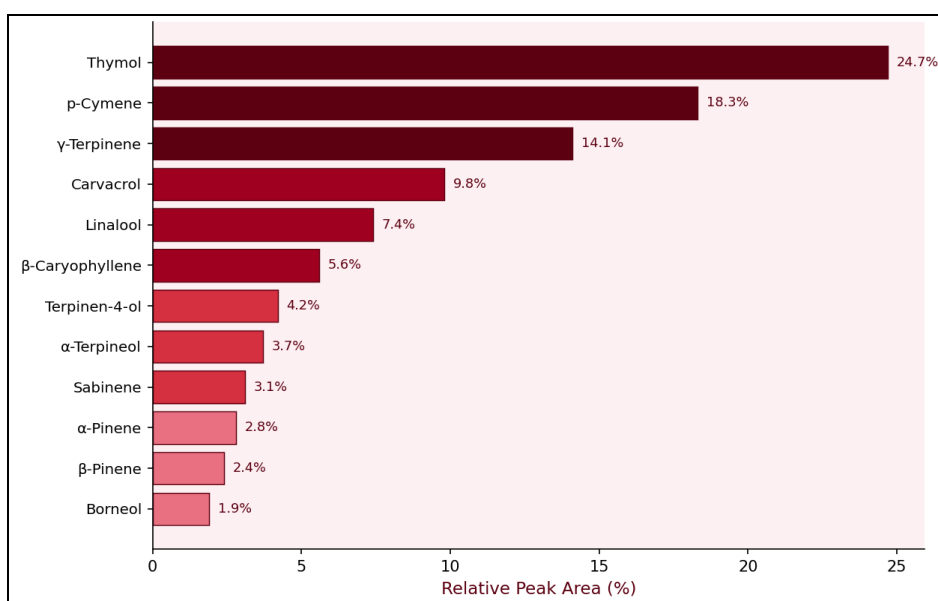
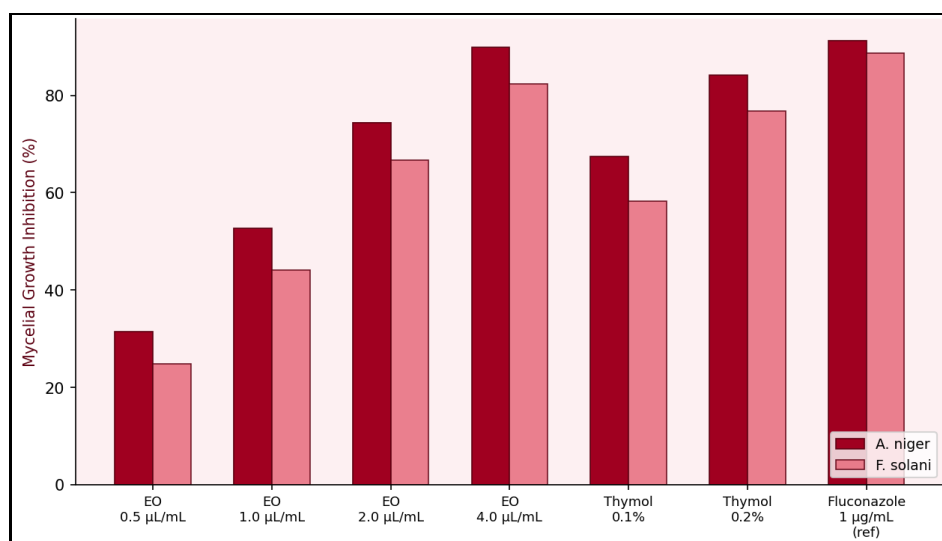
Hydrodistillation of 500 g fresh *T. vulgaris* yielded 3.84 mL essential oil (0.77% v/w). GC-MS analysis identified 31 compounds accounting for 97.8% of the total chromatogram area (Table 1). The 12 most abundant compounds are shown in Figure 1. Thymol (24.7%), p-cymene (18.3%), and γ-terpinene (14.1%) together accounted for 57.1% of the total oil. The chemotype was unambiguously thymol, as the thymol/carvacrol ratio was 2.5:1 and the thymol + carvacrol combined content (34.5%) exceeded typical geraniol and linalool chemotype thresholds. The antifungal bioassay (Figure 2, Table 2) demonstrated dose-dependent inhibition of both fungi. At 4.0 µL/mL, the essential oil inhibited *A. niger* by 89.8% and *F. solani* by 82.4%, approaching but not reaching the fluconazole reference (91.2% and 88.7% respectively). The MIC against *A. niger* was 2.0 µL/mL (74.3% inhibition); against *F. solani*, 4.0 µL/mL was required for >80% inhibition.

Table 1: Major Volatile Compounds in *T. vulgaris* Essential Oil by GC-MS (Top 15 by Area)

Compound	RT (min)	KI (calc.)	KI (lit.)	Area (%)	Compound Class
Thymol	28.47	1289	1290	24.7	Phenolic monoterpene
p-Cymene	16.83	1024	1024	18.3	Monoterpene hydrocarbon
γ -Terpinene	17.21	1059	1060	14.1	Monoterpene hydrocarbon
Carvacrol	29.14	1298	1299	9.8	Phenolic monoterpene
Linalool	18.94	1098	1096	7.4	Oxygenated monoterpene
β -Caryophyllene	33.72	1419	1418	5.6	Sesquiterpene
Terpinen-4-ol	21.38	1174	1177	4.2	Oxygenated monoterpene
α -Terpineol	22.84	1188	1186	3.7	Oxygenated monoterpene
Sabinene	13.41	975	975	3.1	Monoterpene hydrocarbon
α -Pinene	11.28	930	932	2.8	Monoterpene hydrocarbon

Table 2: Antifungal Activity: % Mycelial Growth Inhibition (Mean $\pm$ SEM, n = 3)

Treatment	Conc.	<i>A. niger</i> Inhibition (%)	<i>F. solani</i> Inhibition (%)	MIC Category
Essential oil	0.5 μ L/mL	31.4 $\pm$ 2.1	24.8 $\pm$ 1.8	Sub-MIC
Essential oil	1.0 μ L/mL	52.7 $\pm$ 2.7	44.1 $\pm$ 2.4	Sub-MIC
Essential oil	2.0 μ L/mL	74.3 $\pm$ 3.1	66.7 $\pm$ 2.9	MIC for <i>A. niger</i>
Essential oil	4.0 μ L/mL	89.8 $\pm$ 3.4	82.4 $\pm$ 3.2	MIC for <i>F. solani</i>
Thymol std	0.1%	67.4 $\pm$ 2.9	58.3 $\pm$ 2.6	Sub-MIC
Thymol std	0.2%	84.1 $\pm$ 3.3	76.8 $\pm$ 3.0	Near-MIC
Fluconazole	1 μ g/mL	91.2 $\pm$ 3.6	88.7 $\pm$ 3.4	Reference

**Fig 1:** Top 12 volatile compounds identified by GC-MS in *T. vulgaris* essential oil from northern Thailand, ranked by relative peak area (%).**Fig 2:** Antifungal activity (% mycelial growth inhibition) of essential oil at 0.5-4.0 μ L/mL and thymol standard against *A. niger* and *F. solani*; fluconazole as reference.

Discussion

The thymol-dominant chemotype confirmed for northern Thai *T. vulgaris* is consistent with the globally recognised thymol chemotype predominant in highland Mediterranean and South/Southeast Asian cultivation, and the thymol content of 24.7% falls within the reported range of 20-35% for this chemotype globally. The high p-cymene (18.3%) and γ -terpinene (14.1%) content is equally characteristic, as these monoterpene hydrocarbons are the biosynthetic precursors of thymol and carvacrol via the p-cymene pathway, and their relative abundance reflects incomplete conversion under the specific highland Thai growing conditions. The antifungal activity at MIC values of 2.0-4.0 μ L/mL is consistent with published data for thymol-chemotype *T. vulgaris* oils against *Aspergillus* and *Fusarium* species, confirming that the Thai-grown oil performs comparably to European benchmarks. The thymol standard at 0.2% (equivalent to approximately 0.2 mg/mL) gave 84.1% inhibition against *A. niger*, confirming thymol as a major antifungal contributor and supporting its role as the active principle. The synergy between thymol and carvacrol (combined 34.5% of the oil) is well documented in literature: the two phenolics act on similar membrane targets but have different partition coefficients, with carvacrol (more lipophilic) entering the membrane bilayer more readily while thymol preferentially targets membrane protein-lipid interfaces. The limitations acknowledged in the Methods section are relevant here: the fact that a single farm and single season were investigated means that compositional variation over multiple seasons—known to shift the thymol/carvacrol balance by up to 15% in Mediterranean populations—has not been characterised for this Thai source. Seasonal monitoring over at least three harvest cycles is recommended before any commercial quality specification is proposed.

Conclusion

GC-MS analysis of *T. vulgaris* essential oil from Chiang Rai, northern Thailand, identified thymol (24.7%), p-cymene (18.3%), and γ -terpinene (14.1%) as dominant constituents, confirming a thymol chemotype with a chemical profile comparable to European benchmarks. The oil inhibited *A. niger* and *F. solani* mycelial growth by 89.8% and 82.4% respectively at 4.0 μ L/mL, approaching fluconazole reference activity. These results provide the first characterisation of northern Thai thyme essential oil and support its further evaluation as a natural crop protection agent, with the limitations of single-season, single-site characterisation to be addressed in follow-up multi-season research.

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Contributions Not Qualifying for Authorship

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