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Phytochemical profiling, seed germination responses and *In vitro* propagation of *Thymus serpyllum* L., a medicinal plant of Suru Valley, Ladakh

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Abstract
Thymus serpyllum L. is an aromatic medicinal herb of temperate and high-altitude regions, yet information on populations from the cold-arid region of Ladakh remains limited. This study examined the phytochemical composition, seed germination behaviour and *In vitro* propagation potential of *T. serpyllum* collected from Suru Valley, Kargil. Seed germination was evaluated in a Petri plate bioassay with five pre-sowing treatments using three replicates of ten seeds each. Shade-dried aerial parts were extracted with methanol by Soxhlet extraction and analysed by GC–MS, which revealed 74 chromatographic peaks, of which 25 relatively abundant constituents were examined in detail. For *In vitro* propagation, nodal explants were cultured on Murashige and Skoog (MS) medium supplemented with six combinations of 6-benzylaminopurine (BAP) and kinetin. GA_3 produced the highest germination (73.3%) compared with 23.3% in the control and in the cold + H_2SO_4 treatment, while cold or H_2SO_4 treatment alone gave no germination. The extract contained a chemically diverse mixture of terpenoid, phenolic, fatty-acid-derived, diterpenoid and sterol-related compounds, including thymol (6.60%), 9,12,15-octadecatrienoic acid (6.11%), 4-vinylbenzene-1,2-diol (5.15%), γ-sitosterol (5.11%), *n*-hexadecanoic acid (4.67%) and *p*-cymene-2,5-diol (4.64%). Nodal explants showed only a transient response, with tissue browning preventing sustained shoot proliferation. These findings provide baseline information for the chemical characterisation, seed-based propagation and conservation of *T. serpyllum* from Suru Valley, and indicate that nodal culture requires further optimisation.

Keywords: *Thymus serpyllum*, phytochemical profiling, seed germination, gibberellic acid, *In vitro* culture, Ladakh

Introduction

Medicinal plants have been used for centuries as a source of traditional medicine and continue to contribute to modern healthcare through their naturally occurring bioactive compounds (Salaria *et al*., 2023) [30]. In the Himalayan region, including Ladakh, local communities have long depended on medicinal plants for primary health care, the cold-arid environment of Ladakh, with its high altitude, low rainfall, low temperature and intense solar radiation, provides a distinctive habitat that can influence plant growth and the production of secondary metabolites (Shahar *et al*., 2023) [33].

Thymus serpyllum L., commonly known as creeping or wild thyme, is a perennial aromatic herb of the family Lamiaceae that forms dense mats on rocky slopes, grasslands and open habitats. Its slender creeping stems are woody at the base and bear glandular trichomes that store the essential oils responsible for its characteristic aroma (Jaric *et al*.,2015). The species is widely distributed across Europe, North Africa and Asia (Salaria *et al*., 2023) [30] and in India, occurs mainly in the Western Himalaya, including Jammu & Kashmir, Ladakh, Himachal Pradesh and Uttarakhand (Shahar *et al*., 2023) [33]. It is traditionally used to relieve digestive complaints, is consumed as an herbal tea and serves as a culinary herb. Its nectar-rich flowers also support pollinators, adding to its ecological value (Jalil *et al*., 2024) [18].

The medicinal importance of *T. serpyllum* is attributed to a rich phytochemical composition that includes essential oils, phenolic acids, flavonoids, terpenoids, tannins and glycosides (Jarić *et al*., 2015; Salaria *et al*., 2023) [19, 30]. Thymol and carvacrol are regarded as characteristic constituents of the genus *Thymus*, and *p*-cymene, γ-terpinene, linalool, borneol, β-caryophyllene and α-pinene have been reported from different populations (Satyal *et al*., 2016) [32]. However, the relative proportions of these compounds vary with geographical origin, altitude, climate, developmental stage and genetic background (Ložienė & Venskutonis, 2005; Satyal *et al*., 2016; Jalil *et al*., 2024) [18, 22, 32]. Locally occurring populations should therefore be characterised individually. Soxhlet extraction is widely used to recover phytochemicals from dried plant material because it provides efficient, continuous extraction (Azwanida, 2015) [1], methanol is a preferred solvent for polar and moderately polar metabolites (Dai & Mumper, 2010) [9] and GC–MS is a reliable technique for profiling volatile and semi-volatile constituents of complex plant extracts (Ugwuibe *et al*, 2026) [35].

Sustainable use of medicinal plants also requires reliable propagation methods, particularly because many species are threatened by overexploitation, habitat degradation and climate change (George *et al*., 2008) [14]. Seed germination is the starting point of natural regeneration, but dormancy can prevent germination even under favourable conditions (Finch-Savage & Leubner-Metzger, 2006) [12]. Cold stratification, gibberellic acid (GA_3) and chemical scarification are commonly used to overcome dormancy (Bewley *et al*., 2012) [3] with GA_3 acting by promoting embryo growth and the mobilisation of stored reserves (Nonogaki, 2010) [26]. Plant tissue culture on MS medium (Murashige & Skoog, 1962) [23] offers a complementary route for rapid, season-independent multiplication, and the success of cytokinins such as 6-benzylaminopurine (BAP) and kinetin in shoot induction is well documented (George

et al., 2008) [14]. *In vitro* regeneration of *T. serpyllum* from nodal explants has been reported, although phenolic browning is a recognised obstacle to culture establishment (Khajuria *et al*., 2020).

Most studies on *T. serpyllum* have been conducted on European and other Himalayan populations (Jarić *et al*., 2015; Salaria *et al*., 2023) [19, 30] and to the best of our knowledge, information on seed germination and *In vitro* propagation of the species from Ladakh is lacking, while detailed phytochemical profiling of plants from the Suru Valley of Kargil is insufficient. The present study was therefore undertaken (i) to analyse the phytochemical constituents of the methanolic extract of *T. serpyllum* by Soxhlet extraction followed by GC–MS; (ii) to evaluate the effect of different pre-sowing treatments on seed germination; (iii) to assess the response of nodal explants to different concentrations and combinations of BAP and kinetin.

Materials and Methods

Study area and plant material

The investigation was carried out in the Department of Botany, University of Ladakh, Kargil Campus, Union Territory of Ladakh, India, during 2025 [5]–2026, and comprised a seed germination bioassay, phytochemical extraction followed by GC–MS analysis, and *In vitro* propagation experiments. Fresh aerial parts and mature seeds of *Thymus serpyllum* L. were collected from Sankoo, Suru Valley, Ladakh. Healthy, disease-free plant material was selected and transported to the laboratory in clean polythene bags for further processing.

Seed germination

The effect of pre-sowing (dormancy-breaking) treatments on seed germination was evaluated by a Petri plate bioassay under controlled laboratory conditions, using a completely randomised design with five treatments and three replicates (15 Petri dishes in total). Sterile glass Petri dishes (90 mm diameter) lined with blotting paper were moistened uniformly with sterile distilled water and moisture was maintained throughout the experiment without waterlogging. Healthy, uniform seeds were subjected to the following treatments: T1, control (no pre-treatment); T2, cold stratification at -20 °C for 24 h; T3, GA_3 treatment (seeds soaked in 0.5 mg mL^{-1} GA_3 for 24 h); T4, acid scarification with 70% sulphuric acid (H_2SO_4) for 15 min, followed by repeated washing with sterile distilled water; and T5, cold stratification (-20 °C, 24 h) followed by 70% H_2SO_4 for 15 min and thorough rinsing. Ten seeds were aseptically placed in each Petri dish with sterile forceps, giving 30 seeds per treatment and 150 seeds in total. Dishes were monitored regularly and sterile distilled water was added when required. Seeds were scored as germinated when the radicle had visibly emerged from the seed coat. Germination was recorded daily from the third to the sixth day after sowing, and observations were continued until day 12. Germination percentage was calculated as:

Germination (%) = (number of germinated seeds / total number of seeds sown) × 100

Treatments were compared on the basis of final germination percentage.

Phytochemical analysis

Fresh aerial parts were washed with tap water followed by distilled water to remove dust and contaminants, shade-dried for one month under dust-free conditions until constant weight, powdered with a sterile mortar and pestle, sieved to a fine powder and stored in sterile airtight containers. Approximately 10 g of powder was extracted with 200 mL of methanol in a Soxhlet apparatus at 50 °C for 8–10 h, until the solvent in the siphon tube became colourless. The extract was concentrated under reduced pressure in a rotary evaporator, transferred to sterile centrifuge tubes and stored under refrigeration until analysis. The concentrated extract was analysed at the Central Instrumentation Laboratory, Central University of Punjab (CUPB), Bathinda, using a Shimadzu GCMS-TQ8050 NX equipped with a capillary column. Helium was the carrier gas at a constant flow rate of 1.0 mL min^{-1}, and 1 µL of sample was injected in split mode. The oven temperature was held initially at 70 °C and increased to 310 °C according to the programmed temperature profile. The mass spectrometer was operated in electron ionisation mode over a scan range of m/z 50–700, with a total run time of approximately 63 min. Constituents were tentatively identified by comparing their mass spectra with the National Institute of Standards and Technology (NIST) mass spectral library, and relative abundance was expressed as peak area percentage.

In vitro culture of nodal explants

Healthy, disease-free shoots collected from Suru Valley were processed immediately in the plant tissue culture laboratory. MS basal medium was prepared at 4.43 g L^{-1} with 3% (w/v) sucrose, 0.8% (w/v) agar, 100 mg L^{-1} myo-inositol and 440 mg L^{-1} calcium chloride, and dispensed as 20 mL aliquots into culture tubes. The pH was adjusted to 5.8 ± 0.1 with 1 N NaOH or 1 N HCl, and the medium was autoclaved at 121 °C and 15 psi for 15–20 min. Six treatments (C_1–C_6) were prepared by supplementing the medium with BAP alone or BAP combined with kinetin (Kn).

Explants were washed under running tap water, immersed in Tween-20 solution for a few minutes and rinsed with distilled water. Inside a laminar airflow cabinet they were surface sterilised with 2% sodium hypochlorite for 10 min followed by 0.1% mercuric chloride ($HgCl_2$) for 1 min, rinsed three to four times with sterile distilled water and dipped in 1000 ppm ascorbic acid to reduce tissue browning caused by phenolic compounds. Damaged tissue was trimmed, and explants were inoculated vertically onto the respective media using instruments sterilised with 70% ethanol and flame. Cultures were incubated at 25 ± 2 °C under a 16 h light/8 h dark photoperiod and observed regularly for contamination, explant survival, shoot initiation and shoot multiplication.

Results and Discussion

Seed germination

Germination responses differed markedly among the five pre-sowing treatments (Table 1). No germination occurred in the control, cold, H_2SO_4 or cold + H_2SO_4 treatments during the first three days after sowing, whereas GA_3-treated seeds germinated early. On day 4, limited germination appeared in the control and in the cold + H_2SO_4 treatment, while the cold and H_2SO_4 treatments remained without germination and GA_3 continued to give the highest germination. Germination increased further in the control and cold + H_2SO_4 treatments on days 5 and 6, but seeds in the cold and H_2SO_4 treatments did not germinate at any

point in the experiment. The GA_3 treatment consistently recorded the highest number of germinated seeds and the most vigorous seedling growth. Final germination was highest under GA_3 (73.3%), followed by the control and cold + H_2SO_4 treatments (23.3% each), while cold and H_2SO_4 treatments alone recorded 0% (Table 1). No change in the germination pattern was recorded from day 7 to day 12, indicating that germination had stabilised by day 6; the day-6 data were therefore taken as the final observations.

Table 1: Final germination percentage of *Thymus serpyllum* seeds under different pre-sowing treatments (30 seeds per treatment; three replicates of ten seeds).

Treatment	Description	Germination (%)
T1	Control (no pre-treatment)	23.3
T2	Cold stratification (−20 °C, 24 h)	0.0
T3	GA_3 (0.5 mg mL^{-1}, 24 h)	73.3
T4	Acid scarification (70% H_2SO_4, 15 min)	0.0
T5	Cold stratification (−20 °C, 24 h) + 70% H_2SO_4 (15 min)	23.3

Seed dormancy is a physiological constraint that prevents viable seeds from germinating under otherwise favourable conditions, and its regulation involves interactions between environmental signals and the plant hormones abscisic acid (ABA) and gibberellins (Finch-Savage & Leubner-Metzger, 2006) [12]. The control treatment produced only 23.3% germination, suggesting that a considerable proportion of seeds remained dormant or unable to germinate under the experimental conditions, which is consistent with physiological dormancy (Bewley *et al*., 2012; Baskin & Baskin, 2000) [2, 3].

GA_3 gave by far the highest germination (73.3%), indicating that gibberellin-mediated processes play an important role in overcoming the constraints on germination in *T. serpyllum*. Gibberellins promote the transition from dormancy to germination and act antagonistically to ABA (Finch-Savage & Leubner-Metzger, 2006) [12]; GA signalling promotes embryo growth and the weakening of surrounding tissues, thereby facilitating radicle emergence, and induces hydrolytic enzymes that mobilise stored reserves (Bewley *et al*., 2012) [3]. The early germination and vigorous seedlings in the GA_3 treatment support this interpretation. The result also agrees with the observation that germination of *T. serpyllum* is sensitive to physiological and environmental conditions: Dadach *et al*. (2015) [8] reported that seeds germinated readily under favourable moisture, with germination declining as water stress increased.

The absence of germination after 24 h at −20 °C does not mean that cold treatment cannot promote germination in this species. The effectiveness of stratification depends on temperature, duration and initial dormancy status and many physiologically dormant seeds require prolonged chilling (Baskin & Baskin, 2014) [4]. Seeds in the high-altitude, cold-arid Suru Valley, which experiences pronounced seasonal and diurnal temperature fluctuations (Baqir *et al*., 2025) [5], encounter combinations of temperature, moisture and season rather than a brief exposure to cold, so the short treatment applied here may not have reproduced natural dormancy release. This explanation remains a hypothesis that requires experiments with different temperatures and longer stratification periods.

Similarly, H_2SO_4 scarification alone produced no germination. Chemical scarification mainly overcomes physical dormancy caused by impermeable seed coverings and is less effective where dormancy is primarily physiological (Baskin & Baskin, 2014) [4], so impermeability of the seed coat may not be the main limitation in *T. serpyllum*. The treatment may also have been unsuitable for these small seeds, since excessive scarification can damage seed tissues and reduce viability (Copeland & McDonald, 2012) [6]. The 0% germination should therefore be interpreted as a response to the specific conditions used (70% H_2SO_4, 15 min) rather than evidence that acid scarification can never be effective.

Interestingly, the combined cold + H_2SO_4 treatment produced germination (23.3%) although neither treatment alone did. Although this did not exceed the control, the result suggests that combining physiological conditioning with modification of the seed coverings may have relieved more than one constraint, since dormancy can involve interacting physiological and seed-covering components (Finch-Savage & Leubner-Metzger, 2006; Baskin & Baskin, 2014) [4, 12]. Its limited effectiveness relative to GA_3 indicates that hormonal regulation was more important than seed-coat modification under the conditions tested, and that germination of *T. serpyllum* cannot be explained by a single dormancy-breaking mechanism. The stabilisation of germination by day 6 further suggests that most seeds capable of germinating did so early in incubation (Bewley *et al*., 2012) [3]. Overall, physiological constraints appear to be an important limitation to germination in *T. serpyllum* under the conditions tested and GA_3 offers a useful basis for seed-based propagation and conservation of the species in Suru Valley, where natural regeneration may be limited by the harsh high-altitude environment.

Phytochemical analysis

GC–MS analysis of the methanolic extract revealed 74 chromatographic peaks (Figure 1), corresponding to compounds tentatively identified by comparison with the mass spectral library. The extract was a chemically diverse mixture of terpenoid, phenolic, fatty-acid-derived, diterpenoid, sterol and other volatile or semi-volatile constituents. For detailed interpretation, the 25 compounds with the highest peak area percentages were selected (Table 2) and structures of representative compounds is shown in Figure 2. This selection was based only on relative area and does not imply that the remaining compounds are biologically insignificant.

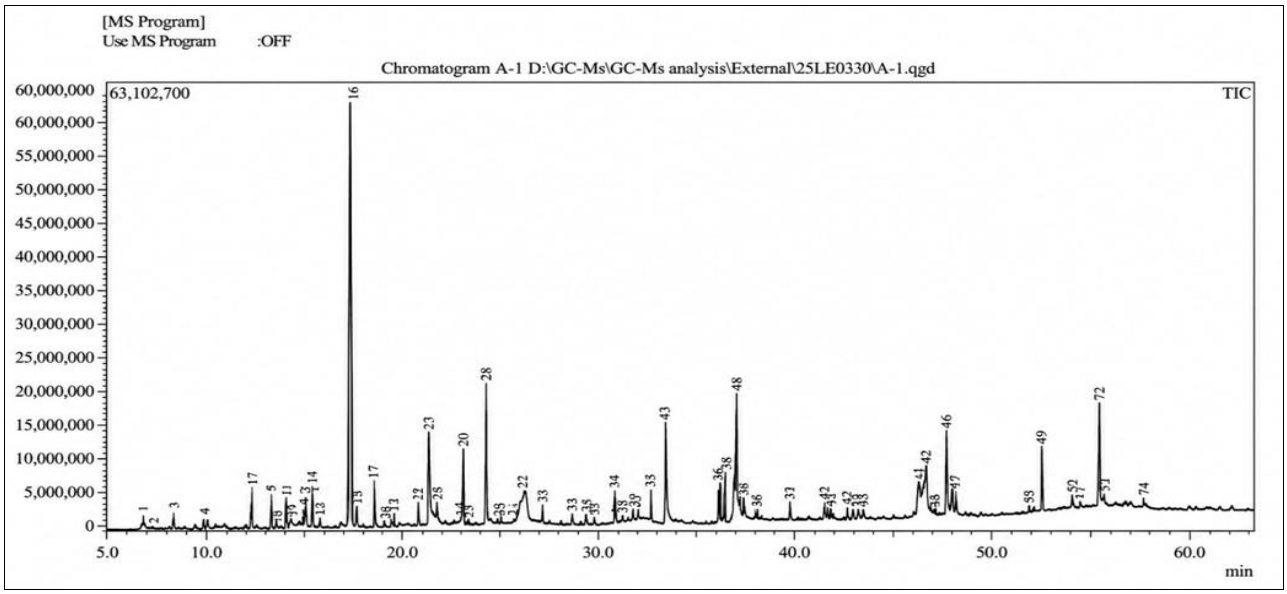


Fig 1: GC–MS total ion chromatogram of the methanolic extract of *Thymus serpyllum* collected from Suru Valley, Ladakh.

Table 2: Major phytochemical compounds identified in the methanolic extract of *Thymus serpyllum* by GC–MS (25 compounds with the highest peak area).

Peak no.	Retention time (min)	Compound identified	Peak area (%)
16	17.07	2,3,5-Trimethylanisole †	22.29
17	17.29	Thymol	6.60
48	37.06	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	6.11
62	46.61	tert-Butylhydroquinone, diacetate	5.42
23	21.34	4-Vinylbenzene-1,2-diol	5.15
72	55.41	γ-Sitosterol	5.11
43	33.54	n-Hexadecanoic acid	4.67
28	24.27	p-Cymene-2,5-diol	4.64
61	46.32	Silane, dimethyl(4-isopropoxyphenoxy)-	3.20
65	47.71	13-Docosenamide, (Z)-	2.81
26	23.11	β-Bisabolene	2.26
69	52.63	Vitamin E	2.11
47	36.91	cis-7-Dodecen-1-yl acetate	1.89
45	36.25	Ethyl oleate	1.29
6	12.27	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-	1.18
10	14.25	Catechol	1.17
19	18.59	Phenol, 5-methyl-2-(1-methylethyl)-	1.10
46	36.46	Phytol	1.08
44	36.12	9,12-Octadecadienoic acid, (Z, Z)-, methyl ester	0.88
7	13.27	Bicyclo [2.2.1] heptan-2-ol, 1,7,7-trimethyl-	0.86
42	32.75	Hexadecanoic acid, methyl ester	0.84
14	15.38	Benzene, 1-methoxy-4-methyl-2-(1-methylethyl)-	0.82
54	41.52	Phenol, 2,3,5,6-tetramethyl-	0.75
9	14.02	α-Terpineol	0.74
12	14.99	5-Hydroxymethylfurfural	0.71

† Tentatively assigned by NIST library match; treated as a possible contaminant or analytical artefact and not considered a confirmed constituent of *T. serpyllum* (see text).

The most abundant peak was 2,3,5-trimethylanisole (22.29%), followed by thymol (6.60%), 9,12,15-octadecatrienoic acid (6.11%), *tert*-butylhydroquinone diacetate (5.42%), 4-vinylbenzene-1,2-diol (5.15%), γ-sitosterol (5.11%), *n*-hexadecanoic acid (4.67%) and *p*-cymene-2,5-diol (4.64%). Other relatively abundant compounds were 13-docosenamide (2.81%), β-bisabolene (2.26%), vitamin E (2.11%) and *cis*-7-dodecen-1-yl acetate (1.89%), while the remaining selected compounds occurred at roughly 0.7–1.4% (Table 2). β-Caryophyllene and cedrol were also detected at lower abundance, and the sterols stigmasterol (0.24%) and campesterol (0.46%) accompanied γ-sitosterol.

The prominence of 2,3,5-trimethylanisole requires cautious interpretation. The most abundant peak, 2,3,5-trimethylanisole (22.29%), was treated as a possible contaminant or analytical artefact and excluded from interpretation. Thus, the reliable metabolite identification requires quality-control procedures that consider sample preparation, data acquisition and background signals (Fiehn *et al*., 2008) [11]. Synthetic or procedure-derived contaminants can appear as major peaks in plant extracts and essential oils and be misinterpreted as native metabolites (Radulović & Blagojević, 2012) [29] and compounds originating from the laboratory environment can interfere with GC–MS analysis (Oca *et al*., 2016) [27].

Because its occurrence may reflect contamination or an artefact introduced during preparation, extraction or analysis, 2,3,5-trimethylanisole was not treated as a confirmed phytochemical constituent of *T. serpyllum* and was excluded from the interpretation below, which provides a more conservative reading of the profile. Analysis of a procedural blank and of replicate extracts would be required to resolve its origin.

Thymol (6.60%) was the second most abundant peak and is the characteristic monoterpene phenol of *Thymus*, associated with the aroma and biological properties of thyme plants. Jarić *et al*. (2015) [19] reported thymol and carvacrol as important monoterpenic phenols of *T. serpyllum*, with composition varying among regions, habitats, developmental stages and climatic conditions. An earlier GC–MS study of Ladakhi *T. serpyllum* reported thymol as the principal constituent (55.98% of the analysed extract), together with compounds such as stigmasterol (Shahar *et al*., 2023) [33]. Our results agree with that study in showing a diverse profile that includes terpenoids and sterols, but the much lower proportion of thymol in the Suru Valley sample indicates a different chemical profile, which may reflect differences in extraction procedure, plant material, environmental conditions or chemotype. Wesołowska *et al*. (2015) [37] listed carvacrol, γ-terpinene, β-caryophyllene, *p*-cymene, (*E*)-β-ocimene and β-bisabolene among the main constituents of *T. serpyllum* essential oil, so the Suru Valley sample contains several compounds previously associated with the species, but in different proportions.

The profile also included considerable fatty-acid-related compounds: 9,12,15-octadecatrienoic acid (6.11%), *n*-hexadecanoic (palmitic) acid (4.67%), ethyl oleate (1.29%), methyl esters of hexadecanoic and 9,12-octadecadienoic acids, and phytol (1.08%). Their detection shows that methanolic extraction recovered lipid-associated constituents in addition to volatile terpenoids; fatty acids and their derivatives have been reported in *Thymus* species, though their abundance depends on the plant material and extraction procedure (Ložienė & Venskutonis, 2005; Gedikoğlu *et al*., 2019) [15, 22]. Phytol is a diterpene alcohol associated with chlorophyll derived material and plant lipid metabolism and has been reported to possess antioxidant and antimicrobial activities (de Moraes *et al*., 2014) [7]. The phenolic fraction comprised 4-vinylbenzene-1,2-diol (5.15%) and *p*-cymene-2,5-diol (4.64%), together with catechol and thymol-related phenols; phenolics are frequently associated with antioxidant properties in aromatic plants, but detection of a compound by GC–MS is not proof of activity in the crude extract (Tohidi *et al*., 2017) [34].

Sterol-related constituents were also prominent, particularly γ-sitosterol (5.11%), with stigmasterol and campesterol at lower levels. Phytosterols are common membrane-associated plant compounds (Piironen *et al*., 2000; Moreau *et al*., 2002) [24, 28], and stigmasterol was also reported among the principal GC–MS constituents of Ladakhi *T. serpyllum* by Shahar *et al*. (2023) [33]. Vitamin E (2.11%) is a lipid-soluble antioxidant produced by photosynthetic organisms (Munné-Bosch & Alegre, 2002) [25], although its identification by library matching should be regarded as tentative. The long-chain fatty amide 13-docosenamide (2.81%) has also been detected by GC–MS in other plant extracts, for example leaf extracts of *Lantana camara* (He *et al*., 2024) [16], but its significance in *T. serpyllum* is unknown. β-Bisabolene (2.26%) and β-caryophyllene indicate a sesquiterpenoid contribution consistent with the chemical polymorphism of *Thymus*; β-caryophyllene is associated with anti-inflammatory and anticancer activities, and chemotypes containing β-bisabolene have been described within a single *Thymus* population (Legault & Pichette, 2007) [21].

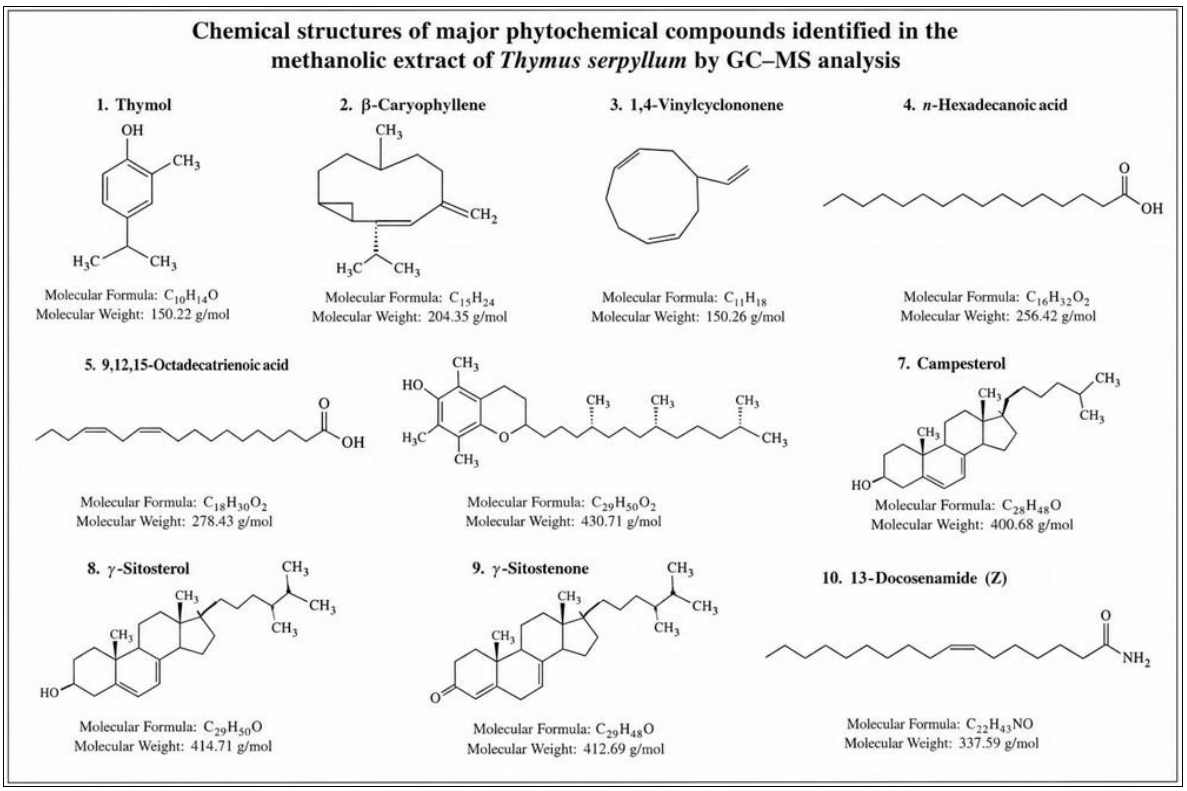


Fig 2: Chemical structures of major phytochemical compounds identified in the methanolic extract of *Thymus serpyllum* by GC–MS analysis.

The predominance of non-thymol constituents differs from many published *T. serpyllum* profiles in which thymol, carvacrol or other monoterpenes dominate. Such differences are not necessarily inconsistent: Ložienė and Venskutonis (2005) [22] found remarkable intraspecific variation in essential oil composition among 26 *T. serpyllum* samples from different habitats and edaphic and climatic factors influence both the quantity and quality of *Thymus* essential oils and the distribution of chemotypes (Vaičiulytė *et al*., 2022) [36]. The distinctive profile of the Suru Valley population may therefore partly reflect adaptation to its high-altitude environment, although this requires comparative sampling of populations from different locations. Most published studies analysed essential oils obtained by hydrodistillation, whereas this study analysed a methanolic Soxhlet extract and differences in solvent polarity and extraction procedure affect which compound classes are recovered; this may explain the appreciable fatty acids, sterols and other less-volatile compounds in the present profile.

Overall, the Suru Valley population of *T. serpyllum* yields a chemically diverse methanolic extract containing terpenoids, phenolics, fatty acids, diterpenoids and sterols. The profile should not be interpreted as evidence that every detected compound is pharmacologically active; GC–MS provides tentative identification and relative chromatographic abundance and confirmation with authentic standards, retention indices or complementary techniques, together with bioactivity testing, would be needed. The data nevertheless provide preliminary chemical information from the Kargil region and a baseline for comparison with other Ladakh populations.

In vitro culture of nodal explants

Nodal explants cultured on MS medium with different concentrations and combinations of BAP and kinetin did not establish a sustained culture. Most explants remained viable for only a short period (7 days) and failed to produce sustained shoot proliferation. In a few cultures initial shoot emergence was observed, but the explants gradually turned brown and failed to develop further. Among the treatments, two explants, each in a separate tube, on MS medium with 2.0 mg L^{-1} BAP + 1.5 mg L^{-1} kinetin (C_6) showed initial shoot development, but the shoots could not maintain growth and deteriorated. Thus, although C_6 induced a temporary shoot response, sustained shoot proliferation and complete plant regeneration were not achieved, and shoot multiplication rate, shoot length, rooting percentage and survival percentage could not be quantified.

Browning followed by growth inhibition was the main limitation. Nodal explants of *T. serpyllum* are known to regenerate on MS medium with suitable growth regulator combinations (Khajuria *et al.* (2020) reported successful shoot induction and multiplication on optimised MS media containing kinetin and BAP, but also highlighted phenolic browning as a difficulty in culture establishment. Phenolic oxidation can compromise explant survival and the ascorbic acid dip used here was evidently insufficient. As success in micropropagation depends on explant type, sterilisation regime, medium, growth regulators and culture conditions (George *et al*., 2008) [14], further work should address browning control

Conclusion

This study provides an integrated preliminary assessment of the phytochemistry, seed germination behaviour and propagation potential of *Thymus serpyllum* from Suru Valley, Ladakh. Seed germination was strongly treatment-dependent: GA_3 gave the highest germination (73.3%), the control and cold + H_2SO_4 treatments gave 23.3%, and cold stratification (24 h) or H_2SO_4 scarification alone gave none, indicating that hormonal stimulation was more effective than the physical and chemical treatments applied. Nodal explants showed only a temporary response, with browning preventing regeneration, whereas seeds germinated and formed seedlings on GA_3-supplemented MS medium, with 1.0 mg L^{-1} GA_3 most effective, identifying seed-based *In vitro* propagation as a promising alternative route for establishing the species. GC–MS analysis of the methanolic extract revealed thymol, 9,12,15-octadecatrienoic acid, 4-vinylbenzene-1,2-diol, γ-sitosterol, *n*-hexadecanoic acid, *p*-cymene-2,5-diol, 13-docosenamide, β-bisabolene and vitamin E were among the notable compounds. The profile differs from that reported for other populations, in keeping with the chemical variability of the species and provides a baseline for future comparative work.

Future work should test a wider range of GA_3 concentrations, longer stratification and modified scarification treatments, include seed viability tests and statistical analysis, develop browning-control strategies and optimised cytokinin combinations for nodal culture, confirm GC–MS identifications with standards and blanks, and evaluate the biological activities of the identified phytochemicals.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

AI Declaration

ChatGPT was used to improve the language and readability of the manuscript.

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