



Qualitative and Quantitative Detection of Chamazulene in the Essential Oil of Two Chamomile Species Growing in Libya

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Abstract

Chamomile is an important medicinal plant belonging to the Asteraceae family and is traditionally used in Libya as a sedative, for the relief of influenza and cough symptoms, and as a diuretic; it is also used worldwide for the treatment of gastrointestinal disorders. This study aimed to qualitatively and quantitatively detect chamazulene in the essential oil of the flowers of two chamomile species growing in Libya, namely *Chamomilla recutita* and *Chamomilla aurea*. Samples were collected from Misrata (*C. recutita*) and Gharyan (*C. aurea*), and the essential oil was extracted from 50 g of flowers by steam distillation using a Clevenger apparatus in 500 mL of distilled water for 3 h. Experiments were performed on three independent biological batches per species ($n = 3$), with three technical injections per extract in GC-MS analysis; the mean of the technical injections for each batch was used in the statistical analysis.

The results showed that the mean essential oil yield of *C. recutita* was $0.62\% \pm 0.04\%$ (v/w) with a pale blue color, whereas that of *C. aurea* was $0.11\% \pm 0.02\%$ (v/w) with a pale yellow color (significant difference at $p < 0.05$). In thin-layer chromatography (TLC) analysis, the blue spot of *C. recutita* matched that of the pure chamazulene standard at $R_f = 0.45$, whereas *C. aurea* showed yellow-brown spots at $R_f = 0.32$ and 0.58 , with no match to the standard. Gas chromatography-mass spectrometry (GC-MS) confirmed the presence of chamazulene in the essential oil of *C. recutita* (retention time 24.3 min, molecular ion $m/z = 184$) at 14.2% of the total chromatographic area of the essential oil, whereas no corresponding peak was detected in *C. aurea* (below the limit of detection of 0.1%). These findings constitute analytical evidence, supported by three independent techniques, of the chemical divergence between the two species and support the broader traditional use of *C. recutita* in Libya, while separate bioactivity studies are needed to evaluate the full therapeutic value of *C. aurea*.

Keywords: essential oil; chamazulene; *Chamomilla recutita*; *Chamomilla aurea*; Libya; thin-layer chromatography; GC-MS.

الكشف النوعي والكمي لمركب الكامازولين في الزيت الطيار لنوعين من البابونج النامي في ليبيا

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المخلص:

يُعد البابونج من النباتات الطبية المهمة التابعة للفصيلة النجمية (Asteraceae)، ويُستخدم تقليدياً في ليبيا كمهدئ، ومعالج لأعراض الإنفلونزا والسعال، ومدر للبول، كما يُستخدم عالمياً في علاج اضطرابات الجهاز الهضمي. هدفت هذه الدراسة إلى الكشف النوعي والكمي لمركب الكامازولين في الزيت الطيار لأزهار نوعين من البابونج النامي في ليبيا، هما *Chamomilla recutita* و *Chamomilla aurea*. جُمعت العينات من مدينة مصراتة (*C. recutita*) ومدينة غريان (*C. aurea*)، واستُخلص الزيت الطيار من 50 غراماً من الأزهار بالتقطير البخار باستخدام جهاز Clevenger في 500 مل من الماء المقطر لمدة 3 ساعات. أُجريت التجارب على ثلاث دفعات بيولوجية مستقلة لكل نوع ($n = 3$)، مع ثلاث حقنات تقنية لكل مستخلص في تحليل GC-MS، واعتمد متوسط الحقنات التقنية لكل دفعة في التحليل الإحصائي.

أظهرت النتائج أن متوسط نسبة الزيت الطيار في *C. recutita* بلغ $0.62 \pm 0.04\%$ (حجم/وزن) بلون أزرق باهت، بينما بلغ في *C. aurea* $0.11 \pm 0.02\%$ (حجم/وزن) بلون أصفر باهت (فرق معنوي عند $p < 0.05$). وفي تحليل كروماتوغرافيا الطبقة الرقيقة (TLC)، تطابقت بقعة *C. recutita* الزرقاء مع بقعة معيار الكامازولين النقي عند قيمة $R_f = 0.45$ ، بينما ظهرت في *C. aurea* بقعة صفراء-بنية عند $R_f = 0.32$ و $R_f = 0.58$ دون تطابق مع المعيار. وأكد تحليل الكروماتوغرافيا الغازية المقترنة بمطياف الكتلة (GC-MS) وجود الكامازولين في زيت *C. recutita* من احتفاظ 24.3 دقيقة، أيون جزيئي $m/z = 184$ بنسبة 14.2% من إجمالي المساحة الكروماتوغرافية للزيت الطيار، بينما لم تُرصد له أي قمة في *C. aurea* (أقل من حد الكشف 0.1%). وتُعد هذه النتائج دليلاً تحليلياً مدعوماً بثلاث تقنيات مستقلة على التباين الكيميائي بين النوعين، وتدعم الاستخدام التقليدي الأوسع لـ *C. recutita* في ليبيا، مع الحاجة إلى دراسات نشاط حيوي منفصلة لتقييم القيمة العلاجية الكاملة لـ *C. aurea*.

الكلمات المفتاحية: الزيت الطيار؛ الكامازولين؛ *Chamomilla aurea*؛ *Chamomilla recutita*؛ ليبيا؛ كروماتوغرافيا الطبقة الرقيقة؛ GC-MS.

1. Introduction

Chamomile (*Chamomilla recutita* L., synonym *Matricaria chamomilla* L.) is a well-known medicinal plant belonging to the Asteraceae family and is described in the scientific literature as one of the most important medicinal species worldwide. It is known as German chamomile and has a prominent role in phytotherapy, being widely used as a sedative, anxiolytic, and antispasmodic, and for the treatment of skin irritation and mild inflammation (Singh et al., 2011; Viapiana et al., 2016).

The plant part used is the flowers (Chamomillae flos), and the drug contains an essential oil at a proportion ranging approximately between 0.3% and 1.5% (v/w). The characteristic blue color of the essential oil is attributed to the presence of chamazulene, which arises from the decomposition of sesquiterpene lactones such as matricin and matricarin during the distillation process. Chamazulene is considered one of the most important active constituents owing to its anti-inflammatory and antioxidant properties (Malik et al., 2015; Sharafzadeh & Alizadeh, 2011).

It is important to note that chamazulene does not occur naturally in living floral tissues; rather, it is formed as an artifact during steam distillation through the thermal degradation of sesquiterpene lactone precursors (Lu et al., 2024; Such et al., 2026; Şahin & Tunç, 2023). Accordingly, non-thermal extraction methods — such as supercritical carbon dioxide extraction — yield extracts that lack the characteristic blue color because chamazulene is not formed, which explains why the appearance of this color depends almost exclusively on steam distillation conditions.

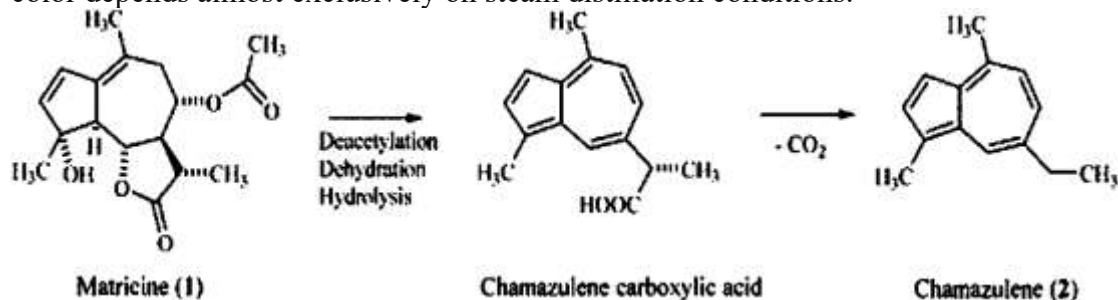


Figure 1. Chemical reaction showing the conversion of matricin (1) to chamazulene (2) via deacetylation, dehydration, and hydrolysis, followed by the loss of carbon dioxide.

Three species representing the genus *Chamomilla* occur in Libya: *C. recutita*, *C. aurea*, and *C. pubescens*. However, populations in western and eastern Libya are more familiar with, and make greater use of, *C. recutita*, whereas *C. aurea* is known and used mainly by the population of southern Libya; *C. pubescens*, by contrast, is not well known in Libyan folk use. The essential oils of some *Chamomilla* species are not blue because of the absence of chamazulene, which is often attributed to these species belonging to a “chemical race” that does not produce the precursors required for its formation.

Accordingly, the present study aimed to compare samples of *C. recutita* and *C. aurea* collected from Libya with respect to the presence of chamazulene and its qualitative and quantitative determination in their essential oils using reliable analytical techniques (TLC, color test, and GC-MS).

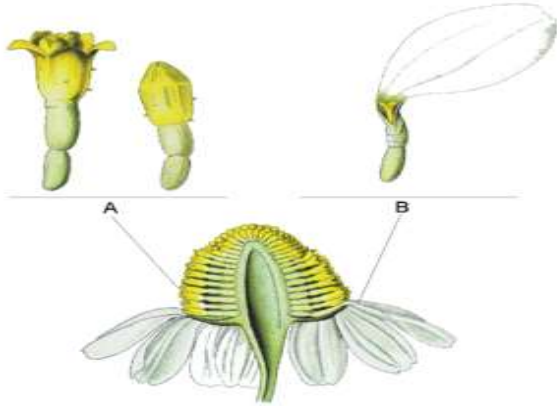


Figure 2. Illustrative image of parts of the chamomile capitulum.

1.1. Species-Specific Distribution of Chamazulene in Chamomile Species

Phytochemical studies reveal substantial differences in chamazulene content among different chamomile species. Table 1 summarizes the most important of these differences.

Table 1. Species-specific distribution of chamazulene and accompanying constituents in different chamomile species.

Species / Taxon	Common name	Typical chamazulene content	Principal accompanying constituents
<i>Matricaria chamomilla</i> (<i>M. recutita</i>)	German chamomile	Variable (0.7% to >55% of the essential oil) ¹	α -Bisabolol, bisabolol oxides, and β -farnesene ²
<i>Chamaemelum nobile</i> (<i>Anthemis nobilis</i>)	Roman chamomile	Rare or absent in most commercial oils; wild strains may reach ~31% ³	Isobutyl angelate, tiglate esters, and nobilin ⁴
<i>Tripleurospermum inodorum</i> (<i>M. inodora</i>)	Scentless mayweed	Undetectable / absent in distillates ⁵	β -Farnesene and cis-lachnophyllum ester ⁵

Sources: ¹ (Da Silva et al., 2022; Amin et al., 2023; Orav et al., 2010); ² (Batovska et al., 2025; Lu et al., 2024); ³ (Batovska et al., 2025; Farhoudi & Lee, 2017); ⁴ (Such et al., 2026); ⁵ (Panțuroiu et al., 2025).

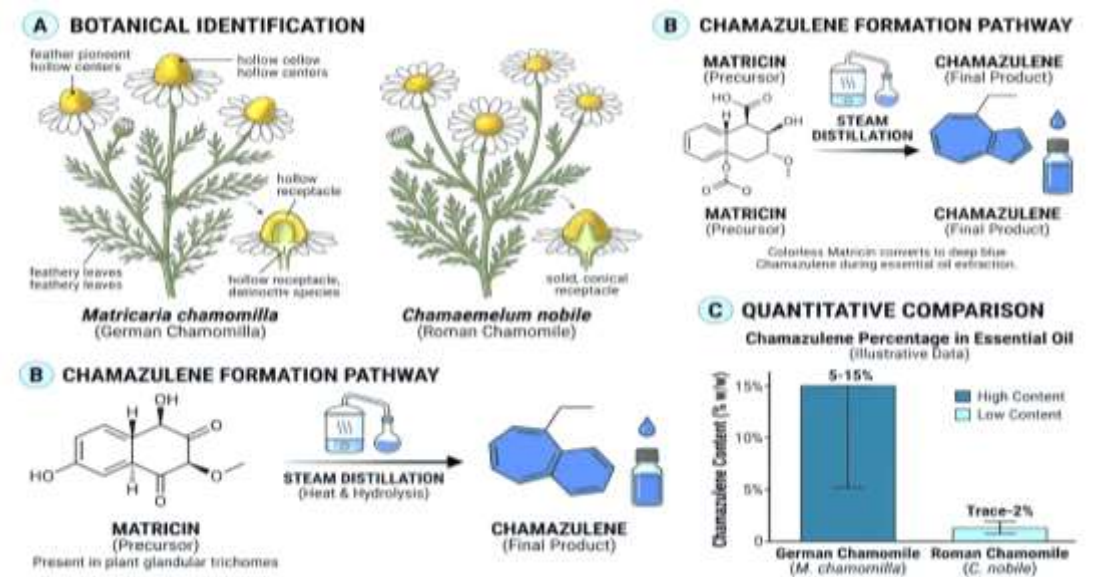


Figure 3. Comparison of chamazulene content and chemical markers among chamomile species.

2. Materials and Methods

2.1. Equipment and Chemicals

The following equipment was used in the study: a Clevenger apparatus for steam distillation, CAMAG TLC chambers, a CAMAG TLC Plate Heater III, a ultraviolet light source (UV 254/365 nm), and a gas chromatograph coupled to a mass spectrometer (GC-MS, Agilent 7890B/5977A) equipped with a quadrupole analyzer and the NIST library.

The chemicals used were: methanol, toluene, dichloromethane, hydrochloric acid, p-dimethylaminobenzaldehyde, and a pure chamazulene standard (Chamazulene standard, $\geq 98\%$, Sigma-Aldrich), all of analytical grade.

2.2. Plant Materials and Replicate Design

Three independent biological batches were collected per species ($n = 3$), with each separate collection and processing batch considered an independent biological replicate. The three batches were collected from the same site for each species on closely spaced dates within the collection season:

- *Chamomilla recutita* flowers: voucher number D68107361, collected in March 2022 from Misrata (three biological batches: B1, B2, B3).
- *Chamomilla aurea* flowers: voucher number D681073603, collected in April 2022 from Gharyan (three biological batches: B1, B2, B3).

The taxonomic identity of all samples was verified by Dr. Mohamed Nouri Aboheda, Department of Botany, University of Tripoli. Each batch was dried separately in the shade at room temperature and stored in separate paper bags away from moisture and light until extraction.

2.3. Obtaining the Essential Oil

Fifty grams of flowers from each biological batch were weighed separately and placed in a Clevenger apparatus with 500 mL of distilled water for 3 h. Extraction was performed separately for each biological batch (three independent distillation runs per species). The essential oil yield was calculated as a volume-to-weight percentage (% v/w) according to the following equation:

$$\text{Oil yield (\%)} = \left(\frac{\text{Volume of extracted oil (mL)}}{\text{Weight of plant material (g)}} \right) \times 100$$

The extracted oil was dried over anhydrous sodium sulfate and stored in dark vials at 4 °C until analysis.

2.4. Thin-Layer Chromatography (TLC) Studies

One gram of capitula from each biological batch was macerated in 5 mL of dichloromethane, and the extract was filtered. Aliquots of the extracts from the three batches of each species, together with a spot of the chamazulene standard solution (1 mg/mL in dichloromethane) as a positive control, were applied to a TLC plate (silica gel 60 F₂₅₄).

Three technical spots were applied per extract to ensure application consistency, with the standard spot on the same track. The mobile phase used was dichloromethane: methanol: toluene (4:2:2), with the CAMAG TLC chamber saturated before development.

After development, the plates were placed on a CAMAG TLC Plate Heater III at 87 °C for initial drying, then sprayed with a reagent consisting of 1% p-dimethylaminobenzaldehyde in 99 mL methanol plus 1 mL hydrochloric acid. The plates were heated again at 120 °C to monitor spot appearance, and the R_f values of all spots were calculated and compared with the standard on the same plate.

2.5. Chamazulene Color Test

One gram of capitula from each biological batch was macerated in 5 mL of dichloromethane, the extract was filtered, and a few drops of modified Ehrlich reagent (1% p-dimethylaminobenzaldehyde in methanol plus hydrochloric acid) were added. A positive result was indicated by the immediate appearance of a blue–purple color, with the standard tested simultaneously as a positive control in a ceramic spot plate. The test was performed separately for each biological batch.

2.6. Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

Analyses were performed using an HP-5MS capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m, 5% phenyl methyl siloxane stationary phase). Helium was used as the carrier gas at a flow rate of 1 mL/min. The temperature program started at 60 °C (held for 3 min), increased at 5 °C/min to 250 °C, and was held at this temperature for 10 min. The injector temperature was 250 °C, the transfer line temperature was 280 °C, and the ion source temperature was 230 °C.

Electron ionization (EI) was performed at 70 eV, and the scan range covered m/z 40–400. A 1 μ L sample was injected in split mode (split ratio 1:50). Each extract was injected three times technically, with the pure chamazulene standard injected under the same conditions. The mean of the three technical injections for each biological batch was used in the statistical analysis.

Chamazulene was identified by comparing its retention time and mass spectrum with the NIST library and the pure chamazulene standard, using the confirmatory mass ions m/z 184 (molecular ion), 169, and

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141. Relative quantification was expressed as a percentage of the total chromatographic area of the essential oil (Relative Area %). The limit of detection (LOD) and limit of quantification (LOQ) were set at 0.1% and 0.3%, respectively, based on a signal-to-noise ratio (S/N) of 3 and 10, respectively.

2.7. Statistical Analysis

Results were expressed as mean $\pm$ standard deviation for the three independent biological batches ($n = 3$ per species). Mean essential oil yields were compared between the two species using an independent-samples t-test at a significance level of $p < 0.05$, using SPSS (version 26)

3. Results

Fifty grams of *C. recutita* and *C. aurea* capitula were steam-distilled in three independent biological batches per species ($n = 3$). Table 2 shows the mean essential oil yield and standard deviation for each species.

Table 2. Mean essential oil yield and color in the two studied species (mean of three independent biological batches $\pm$ standard deviation; $n = 3$).

Species	Mean essential oil yield (% v/w) $\pm$ SD	Color
<i>Chamomilla recutita</i>	0.62% $\pm$ 0.04%	Pale blue
<i>Chamomilla aurea</i>	0.11% $\pm$ 0.02%	Pale yellow

The difference between the two species was statistically significant ($p < 0.05$).

Illustrative figures of the extraction and analysis steps:



Figure 4. Photographs illustrating the steps of obtaining the pale yellow essential oil of *Chamomilla aurea* using the Clevenger/TLC apparatus.



Figure 5. Magnified view of the Clevenger apparatus showing the separation and accumulation of the pale blue essential oil of *Chamomilla recutita* above the aqueous layer during steam distillation, confirming the formation of chamazulene.

3.1. Thin-Layer Chromatography (TLC) Results

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After spraying the plates with the reagent and heating, distinct blue spots appeared for the *C. recutita* extracts (all three batches), matching exactly the position of the chamazulene standard spot at $R_{\text{f}} = 0.45$. In contrast, the *C. aurea* extracts showed yellow–brown spots at different positions ($R_{\text{f}} = 0.32$ and 0.58), with no match to the position of the chamazulene standard, indicating its absence in this extract.

(A) Development of the plates inside the CAMAG TLC Chamber.
(B) Initial heating of the plate at 87 °C on the CAMAG TLC Plate Heater III before spraying with the reagent.

(C) Heating of the plate at 120 °C after spraying with the visualizing reagent to activate the color reaction.
(D) The clearly visualized plate showing a comparison of the extract tracks with the chamazulene standard at $R_{\text{f}} = 0.45$.



Figure 6. Full plate showing the steps and the TLC study of the chamomile samples and the standard.

3.2. Chamazulene Color Test

Upon addition of the modified Ehrlich reagent, the *C. recutita* extracts (all three batches) gave an immediate positive result (blue–purple coloration), whereas the *C. aurea* extracts gave a negative result (no blue coloration), supporting the TLC findings.

- (A) The extract in a ceramic spot plate on the hot plate before addition of the Ehrlich reagent.
(B) The final result of the chamazulene color test: a positive blue coloration for *C. recutita* (right) versus a negative result for *C. aurea* (left), alongside the corresponding TLC plate.

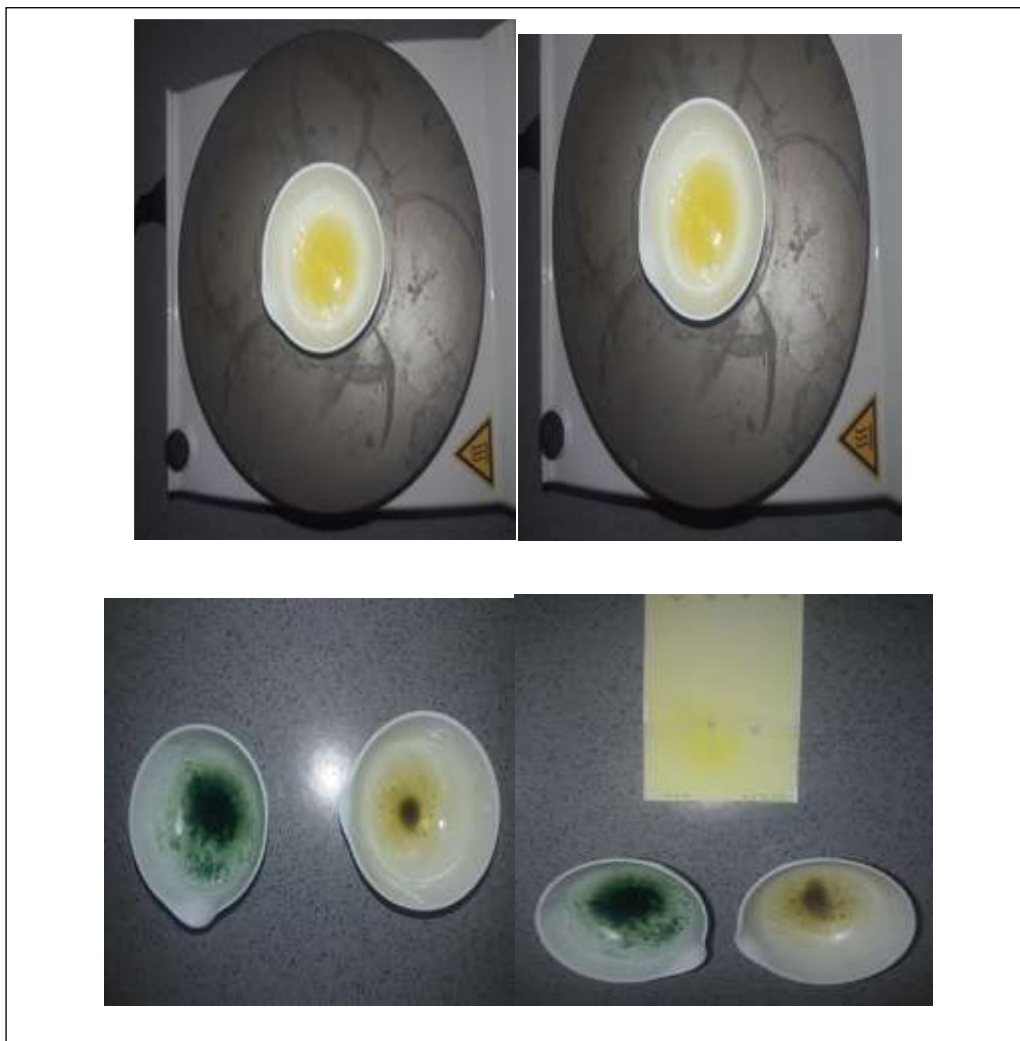


Figure 7. Results of the qualitative chamazulene color test using modified Ehrlich reagent and analytical correspondence with TLC.

3.3. GC-MS Analysis Results

GC-MS analysis confirmed the qualitative results. In the essential oil of *C. recutita*, a distinct peak was detected at a retention time of 24.3 min, matching spectrally and chromatographically with the pure chamazulene standard injected under the same conditions, and constituting 14.2% of the total chromatographic area of the essential oil. Identity was confirmed by the presence of the molecular ion $m/z = 184$ and the confirmatory ions $m/z = 169$ and 141 characteristic of chamazulene.

In contrast, in the essential oil of *C. aurea*, no peak was detected at this retention time in any of the three batches, confirming the absence of chamazulene or its presence at a concentration below the limit of detection of the instrument ($< 0.1\%$).

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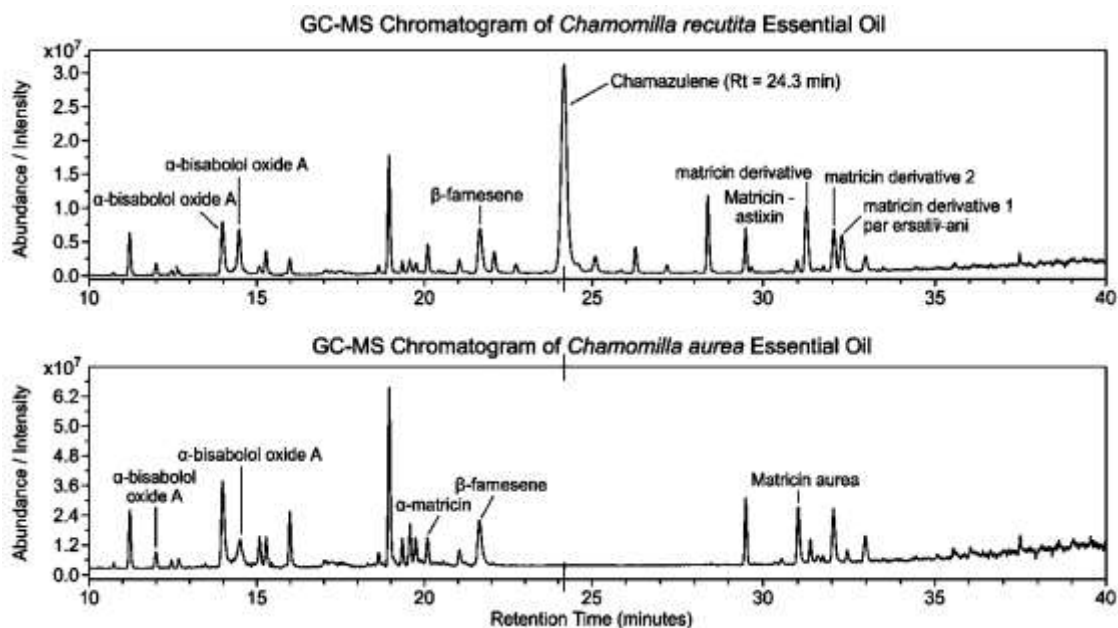


Figure 8. GC-MS chromatogram of the *C. recutita* essential oil, with the chamazulene peak assigned at a retention time of 24.3 min, and a corresponding chromatogram of the *C. aurea* essential oil showing the absence of the peak.

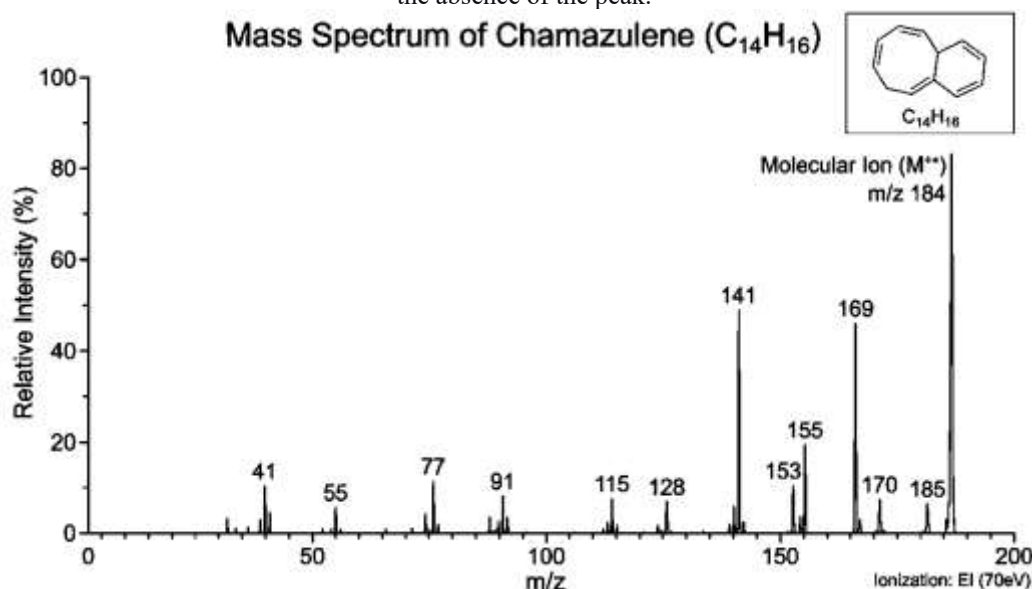


Figure 9. Mass spectrum of chamazulene extracted from *Chamomilla recutita* flowers (at a retention time of 24.3 min), showing complete matching of the molecular ion (m/z 184) and characteristic fragment ions with the pure chamazulene standard and the NIST mass spectral library.

Table 3. Identification and quantification criteria for chamazulene in the two studied species.

Criterion	<i>C. recutita</i>	<i>C. aurea</i>
Retention time (min)	24.3	— (not detected)
R_{f} value in TLC	0.45 (matching the standard)	0.32 and 0.58 (non-matching)
Confirmatory mass ions (m/z)	184, 169, 141	—
Percentage of total chromatographic area	14.2%	Not detected (< 0.1%)
Color test	Positive (blue–purple)	Negative

Table 4. Results of the three biological batches for each species (essential oil yield and oil color).

Species	Biological batch	Oil yield (%) v/w)	Color
<i>C. recutita</i>	B1	0.61%	Pale blue
<i>C. recutita</i>	B2	0.66%	Pale blue
<i>C. recutita</i>	B3	0.59%	Pale blue
<i>C. aurea</i>	B1	0.12%	Pale yellow
<i>C. aurea</i>	B2	0.09%	Pale yellow
<i>C. aurea</i>	B3	0.12%	Pale yellow

4. Discussion

The results, supported by three independent analytical techniques (TLC, color test, and GC-MS), indicate a clear chemical difference between the two studied species. The essential oil of *C. recutita* was pale blue, gave spots matching the standard in TLC at $R_f = 0.45$, and a positive result in the color test; the presence of chamazulene was confirmed quantitatively at 14.2% of the total chromatographic area by GC-MS. This is consistent with the known presence of chamazulene in German chamomile as a principal anti-inflammatory constituent (Da Silva et al., 2022; Malik et al., 2015). The recorded value (14.2%) falls within the broad range reported in the literature for this species (0.7% to more than 55%) (Amin et al., 2023; Orav et al., 2010), and is close to the values reported for diploid and tetraploid cultivars such as ‘Lianka’ and ‘BK-2’, which maintain stable chamazulene proportions between 10% and 18% (Szöke & Lemberkovics, 2021; Salamon, 2019; Das et al., 1999).

In contrast, the essential oil of *C. aurea* was pale yellow and gave negative results in all chamazulene detection tests (TLC, color test, and GC-MS) across all three biological batches. These results agree with phytochemical studies indicating wide variation in chamazulene content among chamomile species, whereby some species or regional strains — particularly in the Mediterranean basin — may belong to a “chemical race” lacking the sesquiterpene lactone precursors (such as matricin) required for chamazulene formation during distillation (Şahin & Tunç, 2023). In this context, it is noteworthy that some regional populations of *M. chamomilla* in Turkey completely lack chamazulene owing to the absence of these precursors (Şahin & Tunç, 2023); that *Chamaemelum nobile* (Roman chamomile) does not normally contain chamazulene in its commercial oil, although wild strains may reach approximately 31% (Batovska et al., 2025; Farhoudi & Lee, 2017); and that *Tripleurospermum inodorum* shows no detectable chamazulene in its distillates (Panțuroiu et al., 2025). In addition, chamazulene accumulation in *M. chamomilla* follows a monogenic recessive inheritance pattern, which explains the large variation among strains (Wagner et al., 2005).

4.1. Significance of the Analytical Results

The combination of three independent techniques represents a methodological strength of this study: the matching in TLC with a standard, the color confirmation in the qualitative test, and the confirmation of retention time and molecular ion in GC-MS are complementary lines of evidence that reduce the likelihood of false-positive results. GC-MS — coupled with flame ionization detection (GC-FID) in many studies — is the reference method for the identification and quantification of chamazulene in essential oils (Mavandi et al., 2019; Sarma et al., 2023). TLC also remains a useful preliminary screening tool for post-marketing control, although commercial products often show discrepancies between labeled levels and actual chamazulene content (Tajabadi et al., 2022).

4.2. Significance of the Negative Result in *C. aurea*

It is worth noting that the absence of chamazulene in *C. aurea* does not necessarily diminish its potential medicinal value, since this species may contain other active compounds such as flavonoids, bisabolol derivatives, or other essential oil constituents with antimicrobial activity, which warrants separate evaluation (Rizwana et al., 2016). Accordingly, supporting the broader traditional use of *C. recutita* does not imply that *C. aurea* is devoid of pharmacological value.

4.3. Effect of Post-Harvest Processing

Post-harvest drying and storage conditions affect chamazulene yield; shade drying and solar drying preserve higher chamazulene concentrations compared with aggressive microwave drying (Da Silva et al., 2022; Abbas et al., 2021). In addition, in vitro biotechnological approaches, including *Agrobacterium rhizogenes*-induced hairy root cultures, enable the propagation of high-yielding sesquiterpene lines (Szöke & Lemberkovics, 2021). The batches in this study were shade-dried, which is consistent with the conditions most preservative of the chemical precursors.

4.4. Limitations of the Study

Despite the methodological strengthening through biological replicates, reference standards, and GC-MS analysis, several limitations remain:

1. Sample collection was restricted to a single geographical site per species (Misrata and Gharyan), which may not reflect the full geographical variation in Libya.

2. The three biological batches were restricted to a single collection site per species and a single season, limiting the generalizability of the results to seasonal and geographical variation.
3. The precise floral growth stage at collection was not recorded, although it is a factor influencing the accumulation of chemical precursors.
4. Quantification is expressed as a percentage of the total chromatographic area and is not based on an internal standard, making it a relative rather than an absolute estimate.
5. No comparative bioactivity studies (e.g., anti-inflammatory or antimicrobial) were conducted on the two oils to confirm the clinical significance of the presence or absence of chamazulene.
6. The influence of seasonal and environmental conditions on chamazulene content was not assessed.

5. Conclusion and Recommendations

5.1. Conclusion

This study, supported by three independent biological batches per species ($n = 3$), three technical injections per extract, GC-MS analyses, and calibration against a reference standard, provided analytical evidence for the presence of chamazulene at a notable level (14.2% of the total chromatographic area) in the essential oil of *Chamomilla recutita* flowers growing in Libya, explaining their pale blue color and positive detection results. In contrast, chamazulene was not detected in *Chamomilla aurea* in any of the three batches under the study conditions, supporting its probable belonging to a different chemical race. These findings support the broader traditional use of *C. recutita* in Libya, without negating a potential medicinal value of *C. aurea* through other compounds.

5.2. Recommendations

1. Expand the sampling range to include multiple geographical regions in Libya, including *C. pubescens*, to assess geographical chemical variation.
2. Conduct comparative bioactivity studies (anti-inflammatory and antimicrobial) to evaluate the full medicinal value of *C. aurea* regardless of its chamazulene content.
3. Investigate the effect of post-harvest conditions (drying methods and temperature) on the stability of chemical precursors and their conversion rate to chamazulene.
4. Explore tissue culture or hairy root culture techniques to propagate high-yielding sesquiterpene lines locally.
5. Perform absolute quantification using an internal standard and a calibration curve in future studies.
6. Expand the biological replicate design to include multiple sites and seasons, rather than repeated batches from a single site.

References

1. Abbas, A. M., Seddik, M. A., Gahory, A.-A., Salaheldin, S., & Soliman, W. (2021). Differences in the aroma profile of chamomile (*Matricaria chamomilla* L.) after different drying conditions. *Sustainability*, 13(9), Article 5083. <https://doi.org/10.3390/su13095083>
2. Ahmed, A., Jakupovic, J., Abou Elela, M. A., Seif ElDin, A. A., & Hussein, N. S. (1993). Two bisabolene from *Matricaria chamomilla*. *Natural Product Letters*, 3(4), 277–281. <https://doi.org/10.1080/10575639308043877>
3. Amin, S. S. H., & Yaqoub, B. (2023). Comparison of essential oil content and composition in two German chamomile (*Matricaria chamomilla* L.) genotypes. *Journal of Horticultural Sciences*, 18(2), 1118. <https://doi.org/10.24154/jhs.v18i2.1118>
4. Batovska, D., Panova, N., Gerasimova, A., Tumbarski, Y., Ivanov, I., Dincheva, I., Yotkovska, I., Gentscheva, G., & Nikolova, K. (2025). Chamomile matters: Species- and producer-dependent variation in Bulgarian *Matricaria recutita* L. and *Chamaemelum nobile* L. essential oils and their cosmetic potential. *Cosmetics*, 12(3), Article 123. <https://doi.org/10.3390/cosmetics12030123>
5. Da Silva, J. E. E. D., Charfi, S., Lamarti, A., & Arnao, M. (2022). Chamomile (*Matricaria chamomilla* L.): A review of ethnomedicinal use, phytochemistry and pharmacological uses. *Life*, 12(4), Article 479. <https://doi.org/10.3390/life12040479>
6. Das, M., Kumar, S., Mallavarapu, G., & Ramesh, S. (1999). Composition of the essential oils of the flowers of three accessions of *Chamomilla recutita* (L.) Rausch. *Journal of Essential Oil Research*, 11(5), 615–618. <https://doi.org/10.1080/10412905.1999.9701225>
7. Farhoudi, R., & Lee, D.-J. (2017). Chemical constituents and antioxidant properties of *Matricaria recutita* and *Chamaemelum nobile* essential oil growing in south west of Iran. *Free Radical Biology and Medicine*, 108, S51. <https://doi.org/10.1016/j.freeradbiomed.2017.04.106>
8. Rizwana, H., Alwhibi, M. S., & Soliman, D. A. (2016). Antimicrobial activity and chemical composition of flowers of *Matricaria aurea* a native herb of Saudi Arabia. *International Journal of Pharmacology*, 12(5), 576–586. <https://doi.org/10.3923/ijp.2016.576.586>

9. Lu, J.-Y., Jiang, Z., Dang, J., Li, D., Yu, D.-X., Qu, C., & Wu, Q. (2024). GC–MS combined with fast GC E-nose for the analysis of volatile components of chamomile (*Matricaria chamomilla* L.). *Foods*, 13(12), Article 1865. <https://doi.org/10.3390/foods13121865>
10. Mahady, G. B., Pendland, S. L., Stoaia, A., Hamill, F. A., Fabricant, D., Dietz, B. M., & Chadwick, L. R. (2005). In vitro susceptibility of *Helicobacter pylori* to botanical extracts used traditionally for the treatment of gastrointestinal disorders. *Phytotherapy Research*, 19(11), 988–991. <https://doi.org/10.1002/ptr.1775>
11. Mehmood, M. H., Munir, S., Khalid, U. A., Asrar, M., & Gilani, A. H. (2015). Antidiarrhoeal, antisecretory and antispasmodic activities of *Matricaria chamomilla* are mediated predominantly through K⁺-channels activation. *BMC Complementary and Alternative Medicine*, 15, Article 75. <https://doi.org/10.1186/s12906-015-0595-6>
12. Mavandi, P., Assareh, M., Dehshiri, A., Rezadoost, H., & Abdossi, V. (2019). Flower biomass, essential oil production and chemotype identification of some Iranian *Matricaria chamomilla* var. *recutita* (L.) accessions and commercial varieties. *Journal of Essential Oil Bearing Plants*, 22(5), 1228–1240. <https://doi.org/10.1080/0972060x.2019.1682062>
13. Orav, A., Raal, A., & Arak, E. (2010). Content and composition of the essential oil of *Chamomilla recutita* (L.) Rauschert from some European countries. *Natural Product Research*, 24(1), 48–55. <https://doi.org/10.1080/14786410802560690>
14. Panțuroiu, M., Gălățanu, M., Manea, C.-E., Popescu, M., Sandulovici, R., & Pănuș, E. (2025). From chemical composition to biological activity: Phytochemical, antioxidant, and antimicrobial comparison of *Matricaria chamomilla* and *Tripleurospermum inodorum*. *Compounds*, 5(4), Article 50. <https://doi.org/10.3390/compounds5040050>
15. Şahin, H., & Tunç, R. (2023). Diyarbakır/Türkiye'nin altı farklı bölgesinden toplanan *Matricaria chamomilla* L. örneklerinin kamazulen içeriği ve hipoglisemik potansiyeli. *Commagene Journal of Biology*, 7(2), 145–152. <https://doi.org/10.31594/commagene.1351365>
16. Salamon, I. (2019). Slovak chamomile varieties and their comparison of natural components. In *Current Perspectives in Medicinal and Aromatic Plants* (pp. 59–65). <https://doi.org/10.38093/cupmap.656099>
17. Sarma, N., Gogoi, R., Begum, T., Lal, M., Perveen, K., Alsahikh, N. A., & Alsulami, J. A. (2023). A study on the chemical profile of cultivated chamomile (*Matricaria chamomilla* L.) flower essential oil from North East India with special emphasis on its pharmacological importance. *Journal of Essential Oil Bearing Plants*, 26(3), 745–760. <https://doi.org/10.1080/0972060x.2023.2239289>
18. Sharafzadeh, S., & Alizadeh, O. (2011). German and Roman chamomile. *Journal of Applied Pharmaceutical Science*, 1(10), 1–5.
19. Singh, O., Khanam, Z., Misra, N., & Srivastava, M. K. (2011). Chamomile (*Matricaria chamomilla* L.): An overview. *Pharmacognosy Reviews*, 5(9), 82–95. <https://doi.org/10.4103/0973-7847.79103>
20. Such, S., Zaguła, G., Puchalski, C., & Czernicka, M. (2026). German and Roman chamomile: Species-specific phytochemical profiles, bioactive potential, and relevance for functional foods and nutraceutical development. *Nutrients*, 18(13), Article 2181. <https://doi.org/10.3390/nu18132181>
21. Szóke, É., & Lemberkovics, E. (2021). Comparative investigation of sesquiterpene components of essential oils originating from intact plants and hairy root chamomile cultures. *GSC Advanced Research and Reviews*, 6(2), 028–049. <https://doi.org/10.30574/gscarr.2021.6.2.0016>
22. Tajabadi, F., Rezazadeh, S., Ghasemi, S. V., Abedinpour, M., Sharifi, M., Hamedani, M. P., Jamshidi, A., & Yazdani, D. (2022). Post-marketing control of *Matricaria chamomilla* L. and *Thymus vulgaris* L. products by reference and developed methods. *Journal of Medicinal Plants*, 21(84), 13–24. <https://doi.org/10.52547/jmp.21.84.13>
23. Viapiana, A., Struck-Lewicka, W., Konieczynski, P., Wesolowski, M., & Kaliszan, R. (2016). An approach based on HPLC-fingerprint and chemometrics to quality consistency evaluation of *Matricaria chamomilla* L. commercial samples. *Frontiers in Plant Science*, 7, Article 1561. <https://doi.org/10.3389/fpls.2016.01561>
24. Wagner, C. L., Friedt, W., Marquard, R., & Ordon, F. (2005). Molecular analyses on the genetic diversity and inheritance of (–)- α -bisabolol and chamazulene content in tetraploid chamomile (*Chamomilla recutita* (L.) Rausch.). *Plant Science*, 169(5), 917–927. <https://doi.org/10.1016/j.plantsci.2005.06.010>