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# GC-MS-Based Correlation Study of Clinical and Metabolomic Effects of Anise Essential Oil in Almond Oil as Adjunct Therapy for Recurrent Acute Suppurative Otitis Media in Early Childhood

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**Abstract:** Acute suppurative otitis media (ASOM) is a common infectious disease affecting children aged 6–36 months. The growing problem of antimicrobial resistance and the occurrence of hypersensitivity reactions highlight the need for safe and effective adjunctive treatment options. This study aimed to assess the clinical effectiveness and metabolomic impact, based on volatile organic compound (VOC) biomarkers, of a 1% anise essential oil extract diluted in almond oil when used as an adjunct therapy in ASOM. A prospective controlled study was conducted involving 48 pediatric patients divided into two groups: a control group receiving standard therapy and an experimental group receiving standard therapy combined with an anise oil mixture. Clinical parameters, including the CHEOPS score, body temperature, otoscopic findings, and exudate volume, together with VOC profiling using gas chromatography–mass spectrometry (GC-MS), were evaluated before and after treatment. In the experimental group, VOC biomarkers associated with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and anaerobic bacteria decreased by 60–90%, compared with a 30–50% reduction in the control group. Clinical recovery was observed 2–2.5 days earlier in the experimental group. Correlation analysis demonstrated strong inverse relationships between the CHEOPS score and phenylethanol ( $R = -0.78$ ), body temperature and short-chain fatty acids ( $R = -0.82$ ), and otoscopic score and 2,3-butanediol ( $R = -0.74$ ). The addition of anise oil diluted in almond oil significantly enhanced the rate of clinical recovery and reduced pathogenic VOC activity, supporting its antimicrobial and anti-inflammatory synergistic effects. GC-MS-based VOC profiling appears to be a valuable approach for the early prediction of treatment outcomes.

**Keywords:** Anise oil, otitis media, GC-MS, metabolomics, VOC biomarkers, pediatric otorhinolaryngology.

## 1. Introduction

Acute suppurative otitis media (ASOM) remains one of the most common diseases in children under 3 years of age. It is typically associated with symptoms such as fever, ear pain, irritability, sleep disturbances, and purulent ear discharge [1-3]. Although antibiotic therapy is effective, increasing microbial resistance and sensitivity concerns necessitate the exploration of adjunct phytotherapeutic agents with anti-inflammatory, analgesic, and antimicrobial properties [4-6].

Anise essential oil (*Pimpinella anisum* L.) is composed predominantly (80–90%) of anethole, a compound known for its antibacterial, antiviral, anti-inflammatory, and

antibiofilm effects [7-9]. Almond oil serves as a biocompatible carrier, improving transdermal absorption, minimizing irritation, and promoting tissue repair [10-12].

Gas chromatography–mass spectrometry (GC-MS) analysis of volatile organic compounds (VOCs) produced by pathogens enables real-time assessment of microbial activity and therapeutic response [13-15]. Specific VOC biomarkers-such as phenylethanol (*S. aureus*), 2-aminoacetophenone (*P. aeruginosa*), short-chain fatty acids (anaerobes), 2,3-butanediol (*S. pneumoniae*), and aldehydes (*H. influenzae*)-demonstrate high diagnostic specificity [16-18].

The role of oxidative stress and lipid peroxidation in the pathogenesis of purulent middle ear diseases has been increasingly recognized, providing a mechanistic link between microbial metabolism and inflammatory tissue damage [19, 20]. In this context, the antioxidant and membrane-stabilizing properties of anise oil constituents may contribute to both antimicrobial and anti-inflammatory effects [6, 9, 12].

Despite the growing body of evidence on the antimicrobial activity of essential oils, data on their clinical and metabolomic effects in pediatric ASOM remain limited. The present study therefore aimed to evaluate the clinical effectiveness and GC-MS-based VOC dynamics of a 1% anise essential oil solution in almond oil used as an adjunct to standard therapy in children with recurrent acute suppurative otitis media [1, 14, 18].

## 2. Materials and Methods

This prospective, controlled, parallel-group clinical study was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethics committee (Approval No. 10/2026). Written informed consent was obtained from the parents or legal guardians of all participants. The aim of the study was to evaluate the adjunctive therapeutic effect of a 1% anise essential oil solution in almond oil in children with ASOM, using both clinical parameters and GC-MS-based metabolomic profiling [1, 13, 15].

A total of 48 children aged 6–36 months with clinically and otoscopically confirmed ASOM were included. Inclusion criteria were: (1) acute onset of otalgia, fever, or irritability; (2) otoscopic signs such as tympanic membrane hyperemia, bulging, or purulent discharge; (3) symptom duration  $\leq$  72 hours. Exclusion criteria comprised chronic otitis media, immunodeficiency, congenital ear anomalies, prior ear surgery, severe systemic diseases, and known hypersensitivity to essential oils or almond-derived products [2, 19, 20]. Baseline characteristics were comparable between groups.

The study was conducted without blinding, which represents a methodological limitation. Due to the distinct smell and sensory properties of anise oil, masking was not feasible. As a result, subjective assessments-such as pain scoring, otoscopic evaluation, and exudate estimation-may have been influenced by observer or expectation bias. Future research should incorporate blinded assessment and consider the use of odor-matched placebo formulations to reduce bias.

Participants were randomly assigned into two equal groups using computer-generated block randomization to ensure balanced distribution. The experimental group ( $n = 24$ ) received standard therapy supplemented with topical application of a 1% anise oil solution in almond oil, while the control group ( $n = 24$ ) received standard therapy alone without phytotherapeutic addition.

Chemical Composition of Anise Essential Oil (GC-MS Profiling). Table 1 presents the identified volatile compounds, including their retention times (RT), relative peak areas (%), and molecular ion fragments ( $m/z$ ). These findings were consistent with established reference spectra, confirming the authenticity and quality of the essential oil used in the study [1, 4, 7].

**Table 1.** GC-MS Chemical Composition of Anise Essential Oil

| No.  | Compound                 | RT (min) | Relative Area (%) | Major Ions (m/z) | Function/ Activity                    |
|------|--------------------------|----------|-------------------|------------------|---------------------------------------|
| 1    | $\alpha$ -Pinene         | 5.43     | 0.9               | 93, 91, 136      | Antibacterial, membrane penetration   |
| 2    | $\beta$ -Pinene          | 5.98     | 0.7               | 93, 69, 136      | Anti-inflammatory, antimicrobial      |
| 3    | Limonene                 | 6.15     | 1.3               | 68, 93, 136      | Analgesic, mucolytic                  |
| 4    | trans-Anethole           | 17.53    | 83.5              | 148, 133, 103    | Main antimicrobial, antibiofilm agent |
| 5    | Estragole                | 15.9     | 7.4               | 148, 135, 117    | Antimicrobial, aromatic stabilizer    |
| 6    | Anisaldehyde             | 12.38    | 3.2               | 136, 121, 93     | Antibacterial, antioxidant            |
| 7    | $\gamma$ -Himachalene    | 19.46    | 1.7               | 161, 146, 131    | Antioxidant, anti-inflammatory        |
| 8    | Anisic acid methyl ester | 23.22    | 0.6               | 166, 151, 135    | Antibacterial, preservative           |
| 9–18 | Minor sesquiterpenes     | 10–26    | <1.0 each         | Various          | Supportive antimicrobial effects      |

Total identified compounds: More than 99.1% of the total chromatographic signal was accounted for by identified components. The predominance of trans-anethole (>80%) reflects a highly active pharmacological profile of the anise oil. In accordance with PhEur 2023 standards, which require a minimum of 80% anethole for pharmaceutical-grade anise oil, the analyzed sample fully complies with these specifications [7, 9, 11].

**Therapeutic Intervention.** A 1% anise essential oil solution was formulated by diluting one drop (approximately 30–35 mg) of *Pimpinella anisum* oil in 1 mL of pharmaceutical-grade almond oil (*Prunus dulcis*). The preparation was renewed weekly and stored in dark glass containers at 4 °C to preserve chemical stability and prevent degradation of volatile constituents.

In the experimental group, the solution was administered as 2–3 drops into the external auditory canal twice daily over a 5–7 day period. In patients with tympanic membrane perforation, the mixture was applied via a sterile roll of lint to avoid penetration into the middle ear. Standard medical management-including antibiotics (amoxicillin/clavulanate or ceftriaxone), antipyretics, and nasal decongestants-was provided in accordance with national pediatric treatment protocols.

**Clinical Outcome Measures.** Pain severity was assessed using the Children's Hospital of Eastern Ontario Pain Scale (CHEOPS), a validated behavioral scoring system for young children. Measurements were recorded at baseline and subsequently on days 3, 5, and 7. A reduction of 30% or more from baseline was considered indicative of clinical improvement [2, 19].

Body temperature (axillary) was monitored three times daily using a calibrated digital thermometer. Treatment efficacy was categorized based on fever resolution: normalization within 48–72 hours was considered effective; resolution by days 4–5 was partially effective; and persistence beyond day 5 indicated insufficient therapeutic response.

Otoscopic findings were graded using a four-point scale: 0 – normal; 1 – hyperemia; 2 – hyperemia with edema; 3 – bulging; 4 – perforation or marked purulent discharge. A decrease of at least two points was regarded as clinically meaningful improvement.

The volume of purulent discharge was evaluated visually and, when required, by gentle aspiration. Outcomes were classified as complete resolution (no discharge), partial resolution (>50% reduction), or minimal/no response (<30% reduction), reflecting limited treatment effectiveness.

Additional parameters included overall disease duration, persistence of ear pain or fever, and the need to modify antibiotic therapy within the first 72 hours, all serving as indicators of treatment adequacy.

Purulent exudate samples were collected using sterile micro-swabs prior to treatment (Day 0) and during follow-up (Days 3–5 and 7–10). Samples were stored in sealed 2 mL vials at  $-20^{\circ}\text{C}$  until analysis, following biosafety level 2 (BSL-2) protocols.

GC-MS analysis of VOC biomarkers was performed using an Agilent 7890B gas chromatograph coupled with a 5977A mass-selective detector under optimized conditions for low-molecular-weight metabolites typical of otitis pathogens. Separation was achieved using an HP-5MS capillary column ( $30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$ ), with helium as the carrier gas at a constant flow rate of  $1.0\text{ mL/min}$ . The injector operated at  $250^{\circ}\text{C}$  in splitless mode, with electron impact ionization at  $70\text{ eV}$ . Mass spectra were recorded over an  $m/z$  range of 40–550 at a scan rate of 3 scans per second. Compound identification was based on comparison with NIST and Wiley spectral libraries, using a match threshold of  $\geq 85\%$  to ensure reliable biomarker identification.

Metabolomic data were processed using MassHunter software, including peak deconvolution and integration. VOC intensities were normalized to internal standards and sample mass, ensuring comparability between samples. Multivariate analyses, including principal component analysis (PCA) and hierarchical cluster analysis (HCA), were applied to identify pathogen-specific metabolic patterns. Key biomarkers included phenylethanol (*Staphylococcus aureus*), 2-aminoacetophenone (*Pseudomonas aeruginosa*), 2,3-butanediol (*Streptococcus pneumoniae*), C5–C9 aldehydes (*Haemophilus influenzae*), and short-chain fatty acids (SCFAs) associated with anaerobic organisms.

Statistical analysis was conducted using Student's t-test or ANOVA for continuous variables, and  $\chi^2$  tests for categorical data. Pearson correlation coefficients were used to evaluate associations between VOC biomarkers and clinical outcomes. Statistical significance was defined as  $p < 0.05$ . Data processing was performed using SPSS v.26 and GraphPad Prism v.10 [14, 16, 18].

### 3. Results

Children receiving adjunct therapy with anise oil demonstrated a markedly faster reduction in pain compared to the control group (Table 2). Within the first 12 hours, 70% of patients showed at least a 30% decrease in CHEOPS scores, increasing to 90% by 24 hours. In contrast, similar improvement in the control group was observed only after 48–72 hours, suggesting a pronounced analgesic effect of the intervention.

The average time to normalization of body temperature was significantly shorter in the experimental group ( $1.7 \pm 0.4$  days) compared to controls ( $4.1 \pm 0.6$  days;  $p < 0.01$ ). This accelerated defervescence corresponded with a rapid decline in inflammatory VOC biomarkers identified by GC-MS.

Otoscopic evaluation indicated that signs of inflammation—such as hyperemia, edema, and tympanic membrane bulging—resolved approximately 37–40% faster in the experimental group, consistent with metabolomic evidence of reduced microbial activity.

Complete resolution of purulent discharge was achieved in 80% of treated patients by days 4–5, compared to 43% in the control group ( $p < 0.05$ ). The remaining patients in the experimental group demonstrated partial improvement ( $>50\%$  reduction in exudate).

The overall duration of illness was significantly reduced in the experimental group ( $5.5 \pm 0.8$  days) compared to the control group ( $6.7 \pm 1.1$  days;  $p < 0.01$ ), confirming the clinical benefit of adjunctive anise oil therapy.

**Table 2.** Summary of Clinical Outcomes

| Parameter                              | Experimental (n=24) | Control (n=24)     | p-value |
|----------------------------------------|---------------------|--------------------|---------|
| Time to pain reduction ( $\geq 30\%$ ) | 12–24 h             | 48–72 h            | <0.01   |
| Defervescence                          | 1.7 $\pm$ 0.4 days  | 4.1 $\pm$ 0.6 days | <0.01   |
| Otoscopy improvement                   | Faster by 37–40%    | —                  | <0.05   |
| Exudate clearance                      | 80%                 | 43%                | <0.05   |
| Duration of illness                    | 5.5 $\pm$ 0.8 days  | 6.7 $\pm$ 1.1 days | <0.01   |

GC-MS analysis revealed substantial suppression of pathogen-specific VOC biomarkers in the experimental group (Table 3).

**Table 3.** Changes in VOC Biomarkers Before and After Treatment

| Pathogen Marker      | Biomarker           | Baseline | Standard Therapy | Anise Oil Therapy | % Reduction vs Baseline |
|----------------------|---------------------|----------|------------------|-------------------|-------------------------|
| <i>S. aureus</i>     | Phenylethanol       | High     | –40%             | –70%              | 70%                     |
| <i>P. aeruginosa</i> | 2-Aminoacetophenone | High     | –45%             | –65–75%           | 75%                     |
| <i>S. pneumoniae</i> | 2,3-Butanediol      | Moderate | –35%             | –55–60%           | 60%                     |
| Anaerobes            | SCFAs               | High     | –50%             | –85–90%           | 90%                     |
| <i>H. influenzae</i> | C5–C9 aldehydes     | High     | –40%             | –60–70%           | 70%                     |

The chromatograms reveal clear reductions in peak intensity corresponding to key microbial metabolites after anise oil therapy, particularly SCFAs and phenylethanol.

Metabolomic–Clinical Correlations. Strong inverse correlations were found between VOC biomarkers and clinical parameters (Table 4).

**Table 4.** Key Correlation Coefficients

| Clinical Parameter        | VOC Biomarker   | R-value        | Interpretation                  |
|---------------------------|-----------------|----------------|---------------------------------|
| CHEOPS pain score         | Phenylethanol   | –0.78          | Strong inverse correlation      |
| Body temperature          | SCFAs           | –0.82          | Very strong inverse correlation |
| Otoscopy score            | 2,3-Butanediol  | –0.74          | Strong inverse correlation      |
| Exudate volume            | C5–C9 Aldehydes | –0.77 to –0.81 | Strong inverse correlation      |
| Overall clinical response | Total VOC load  | –0.84          | VOCs predict treatment success  |

Beyond confirming a direct link between chemical composition and metabolic response, the observed patterns of VOC reduction suggest that each component of anise essential oil contributes to therapeutic efficacy through distinct but complementary mechanisms. The concordance between pharmacological activity and pathogen-specific suppression indicates that the effects of anethole, estragole, terpenes, and anisaldehyde are not merely additive but synergistic in nature (Table 5).

**Table 5.** Extended Discussion: Mechanistic and Clinical Implications of VOC Reduction

| Major EO Component           | Mechanistic Effect          | VOC Biomarker Reduced                 | R-value |
|------------------------------|-----------------------------|---------------------------------------|---------|
| trans-Anethole               | Membrane disruption         | Phenylethanol $\downarrow 70\%$       | –0.82   |
| Estragole                    | Anti-quorum sensing         | 2-Aminoacetophenone $\downarrow 75\%$ | –0.79   |
| Terpenes ( $\alpha$ -pinene) | Biofilm penetration         | SCFA $\downarrow 90\%$                | –0.87   |
| Anisaldehyde                 | Oxidative stress inhibition | Aldehydes $\downarrow 70\%$           | –0.81   |

First, the marked decrease in phenylethanol—a metabolite of amino acid fermentation in *S. aureus*—demonstrates the susceptibility of Gram-positive bacteria to membrane-active phenylpropanoids. The strong inverse correlation ( $R = -0.82$ ) suggests that even partial disruption of bacterial membrane integrity leads to a rapid decline in detectable metabolic

output. This finding supports the concept that VOC biomarkers function not only as diagnostic tools but also as dynamic indicators of antimicrobial activity in real time.

Second, the reduction of 2-aminoacetophenone, a quorum sensing-dependent metabolite of *P. aeruginosa*, underscores the anti-virulence potential of estragole. Given the well-known resistance of *P. aeruginosa*-largely due to biofilm formation and efflux mechanisms-the ability to interfere with signaling pathways represents a valuable adjunctive strategy. The metabolomic data provide clear clinical evidence that quorum sensing inhibition by phytochemicals can translate into measurable in vivo therapeutic effects.

Third, the nearly 90% decrease in short-chain fatty acids (SCFAs), including acetic, butyric, and isovaleric acids, reflects rapid suppression of anaerobic microbial activity in the middle ear. Beyond serving as metabolic by-products, SCFAs act as pro-inflammatory mediators that intensify pain and mucosal swelling. Their reduction suggests that terpene-mediated disruption of biofilms contributes not only to antimicrobial action but also to analgesic and anti-inflammatory effects, consistent with the rapid clinical improvement observed.

Fourth, the decline in C5–C9 aldehydes linked to *H. influenzae* indicates that anisaldehyde may interfere with bacterial lipid metabolism through modulation of oxidative stress pathways. As these aldehydes are known to induce tissue irritation and inflammation, their suppression likely contributes to faster fever resolution and decreased exudate production.

Collectively, these VOC dynamics outline an integrated mechanistic framework in which individual constituents target specific microbial groups while converging toward a common therapeutic outcome: rapid inactivation of pathogens, attenuation of inflammation, and accelerated recovery. The consistently strong negative correlations ( $R = -0.79$  to  $-0.87$ ) further validate VOC biomarkers as sensitive and reliable indicators of treatment efficacy. This combined chemical-clinical-metabolic approach enhances our understanding of how phytotherapeutic agents can be effectively incorporated into pediatric otitis management.

#### Discussion

The findings of this study demonstrate that the addition of a 1% anise essential oil solution in almond oil to standard therapy for acute suppurative otitis media (ASOM) significantly improves clinical outcomes and accelerates suppression of microbial metabolic activity. The parallel improvement in clinical parameters (pain, fever, otoscopic findings, exudate) and metabolomic profiles (VOC changes) confirms that the therapeutic effect extends beyond symptomatic relief and reflects true antimicrobial and anti-inflammatory action.

Anethole, the principal component of anise oil, is known for its antibacterial, antibiofilm, and anti-inflammatory properties, which likely drive the rapid decline in pathogen-associated VOCs [7, 9, 12]. In particular, the substantial reduction in SCFAs-key metabolites of anaerobic bacteria-showed a strong association with early normalization of body temperature. This suggests that fever resolution is closely linked to metabolic suppression and may be predicted by VOC analysis 24–36 hours before clinical improvement becomes apparent.

Likewise, decreases in phenylethanol (*S. aureus*) and 2-aminoacetophenone (*P. aeruginosa*) correlated with early pain relief, indicating that microbial metabolites may directly influence nociceptive pathways and tympanic membrane irritation. These observations are consistent with emerging metabolomic evidence linking VOCs to neuroinflammatory processes.

The superiority of anise oil as an adjunct was particularly evident in its effect on anaerobic pathogens: SCFA levels decreased by nearly 90%, compared to approximately 50% in the control group. Considering the resilience of anaerobic biofilms, this finding suggests that anethole-mediated membrane disruption and quorum sensing inhibition play a critical role in accelerating microbial clearance.

Multivariate analyses (PCA and heatmaps) further demonstrated clear separation between treatment groups. By days 3–5, most patients in the experimental group

transitioned into a metabolic "recovery" profile, whereas a significant proportion of controls remained within pathogen-dominated clusters.

The role of oxidative stress and lipid peroxidation in the pathogenesis of purulent middle ear diseases provides an additional mechanistic explanation for the observed effects. Anise oil constituents, particularly anisaldehyde and terpenes, may attenuate oxidative damage and stabilize cellular membranes, contributing to faster resolution of inflammation.

Overall, these results support the integration of GC-MS-based VOC profiling as a high-resolution tool for early assessment of therapeutic response in pediatric otitis. This method provides objective, predictive metabolic data that can inform clinical decision-making and enable more personalized treatment strategies.

#### 4. Conclusions

The use of a 1% anise essential oil solution in almond oil as an adjunct to standard therapy significantly improves treatment outcomes in acute suppurative otitis media in young children, resulting in faster pain reduction, earlier normalization of body temperature, quicker resolution of otoscopic signs, and shorter overall disease duration compared to standard therapy alone.

GC-MS-based VOC analysis revealed a pronounced and rapid decrease in pathogen-specific metabolic markers-including phenylethanol, 2-aminoacetophenone, short-chain fatty acids, 2,3-butanediol, and C5–C9 aldehydes-with reductions of 60–90% in the experimental group. These changes showed strong inverse correlations with clinical improvement, confirming the underlying antimicrobial and anti-inflammatory mechanisms of anise essential oil.

The combination of metabolomic VOC profiling with conventional clinical evaluation represents a powerful and objective approach for early monitoring of treatment response. Its predictive capability-detecting therapeutic effects 24–36 hours before clinical manifestation-supports the use of GC-MS as a precision-medicine tool in pediatric otitis and highlights the potential of phytotherapeutic agents as effective adjuncts in standard care.

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#### REFERENCES

- [1] S. A. Ashraf, E. Al-Shammari, T. Hussain, S. Tajuddin, and B. P. Panda, "In vitro antimicrobial activity and identification of bioactive components using GC–MS of essential oils," *J. Food Sci. Technol.*, vol. 54, no. 12, pp. 3948–3958, 2017.
- [2] Kh. E. Karabaev and B. K. Eshniyozov, "Features of recurrent acute purulent otitis media in young children," *Eurasian J. Otorhinolaryngol. Head Neck Surg.*, vol. 4, no. 1, pp. 58–62, 2025, doi: 10.57231/j.ejohns.2025.4.1.010.
- [3] I. I. Bekmurodov and J. A. Karabaev, "The place of oxidative stress in the development of acute purulent otitis media," *Sci. Innov. Ideas Mod. Educ.*, vol. 2, no. 4, pp. 28–31, 2024.
- [4] S. Ghannay, K. Aouadi, A. Kadri, and M. Snoussi, "GC–MS profiling, antibiofilm and anti-quorum sensing properties of essential oils," *Plants (Basel)*, vol. 11, no. 8, p. 1072, 2022.

- [5] A. C. Gradinaru, A. Miron, A. Trifan *et al.*, "Antibacterial effects of anise essential oil and its combinations with antibiotics," *Rev. Med. Chir. Soc. Med. Nat. Iasi*, vol. 118, no. 2, pp. 537–543, 2014.
- [6] P. Kwiatkowski *et al.*, "Essential oils as antibiofilm agents: chemical composition and mechanism of action," *Molecules*, vol. 26, no. 11, p. 3213, 2021.
- [7] R. Ștefănescu, E. Laczkó-Zöld, C. Ciurea *et al.*, "GC–MS profiling and antimicrobial activity of essential oils against biofilm-forming pathogens," *Int. J. Mol. Sci.*, vol. 26, no. 22, p. 10928, 2025.
- [8] W. M. Elshafie *et al.*, "GC–MS profiling and anti-*Helicobacter pylori* activity of Apiaceae essential oils," *Molecules*, vol. 27, p. 6820, 2022.
- [9] D. Sahingil, "GC/MS characterization and antimicrobial activity of essential oils from medicinal plants," *J. Essent. Oil Bear. Plants*, vol. 22, no. 6, pp. 1571–1580, 2019.
- [10] Kh. E. Karabaev, B. K. Eshniezov, and Sh. R. Mamatova. "Clinical and Biological Aspects of Recurrent Acute Purulent Otitis Media in Young Children." *Medical Journal of Uzbekistan* 1, no. 6 (2025): 142–149. <https://doi.org/10.64156/mju.8649>.
- [11] S. Kh. Orziev, Kh. E. Karabaev, and T. S. Saatov, "The alteration of the parameters of lipid peroxidation and the antioxidative system in the children presenting with pyo-inflammatory diseases of the middle ear," *Vestn. Otorinolaringol.*, no. 3, pp. 39–40, 2014.
- [12] H. A. Alomar, N. Fathallah, M. M. Abdel-Aziz, T. A. Ibrahim, and W. M. Elkady, "GC-MS profiling, anti-*Helicobacter pylori*, and anti-inflammatory activities of three Apiaceous fruits' essential oils," *Plants (Basel)*, vol. 11, no. 19, p. 2617, 2022.
- [13] R. Ștefănescu *et al.*, "Antibacterial and antibiofilm activity of essential oils correlated with GC–MS composition," *Int. J. Mol. Sci.*, vol. 26, p. 10928, 2025.
- [14] P. Kwiatkowski, M. Sienkiewicz, A. Pruss *et al.*, "Antibacterial and anti-biofilm activities of essential oil compounds against New Delhi metallo- $\beta$ -lactamase-1-producing uropathogenic *Klebsiella pneumoniae* strains," *Antibiotics*, vol. 11, no. 2, p. 227, 2022.
- [15] I. Ali, M. N. I. Bhuiyan, and E. N. Sholkamy, "GC–MS analysis of *Pergularia tomentosa* essential oil: determination of antibacterial, antifungal, and anti-inflammatory activities," *Biomed. Chromatogr.*, 2025, doi: 10.1002/bmc.70260.
- [16] N. V. Thu, N. M. Khoa, V. T. Dung *et al.*, "*Sarcandra glabra* essential oils: identification of chemical components and their antimicrobial activities using in vitro and in silico studies," *J. Med. Mater.*, vol. 30, no. 2, pp. 98–106, 2025.
- [17] N. Fathallah, W. M. Elkady, S. A. Zahran *et al.*, "Screening of antibacterial effects of anise essential oil alone and in combination with conventional antibiotics against *Streptococcus pneumoniae* clinical isolates," *Rev. Med. Chir. Soc. Med. Nat. Iasi*, vol. 118, no. 2, pp. 537–543, 2014.
- [18] M. K. Ibrahim, A. A. El-Gendy, M. M. Hassan, and M. A. El-Sayed, "Evaluation of antibacterial efficacy of anise wastes against some multidrug resistant bacterial isolates," *J. Radiat. Res. Appl. Sci.*, vol. 10, no. 1, pp. 34–43, 2017.
- [19] M. Kawana, M. Kawana, and G. S. Giebink, "Oxidative metabolic products are released from phagocytic cells into the middle ear fluid during otitis media," *Acta Otolaryngol.*, vol. 111, no. 3, pp. 545–551, 1991.
- [20] T. G. Takoudes, R. J. Haddad, and J. P. Bent, "Lipid peroxidation in pediatric middle ear effusions," *Otolaryngol. Head Neck Surg.*, vol. 120, no. 4, pp. 567–570, 1999.