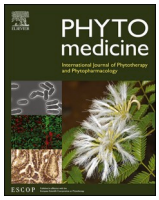
Phytomedicine 156 (2026) 158222

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed



Original Article

Adjuvant treatment with an oleuropein-enriched olive leaf extract improves periodontal outcomes in older adults with periodontitis: Metabolomic insights from a randomized controlled trial

Tamara Y. Forbes-Hernández [a,1], Franklin G. Vargas-Corral [a,1], Beatriz Bullón [b], Lorenzo Rivas-García [a], Vivian Lipari [c], Francesca Giampieri [c,d,e,f], Giuseppe Grosso [g], Maurizio Battino [c,d,e,h], Pedro Bullón [b], José L. Quiles [a,c,*]

[a] Department of Physiology, Institute of Nutrition and Food Technology "José Mataix Verdú", Biomedical Research Centre, University of Granada, 18016 Armilla, Spain
[b] Department of Stomatology, Dental School, Universidad de Sevilla, Sevilla, Spain
[c] Research Group on Foods, Nutritional Biochemistry and Health, Universidad Europea del Atlántico, Isabel Torres, 21, 39011 Santander, Spain
[d] Department of Clinical Sciences, Polytechnic University of Marche, Via Pietro Ranieri 65, Ancona, Italy
[e] Joint Laboratory on Food Science, Nutrition, and Intelligent Processing of Foods, Polytechnic University of Marche, Italy, Universidad Europea del Atlántico Spain and Jiangsu University, China at the Polytechnic University of Marche, Italy
[f] International Research Center for Food Nutrition and Safety, Jiangsu University, Zhenjiang, China
[g] Department of Biomedical and Biotechnological Sciences, University of Catania, 95123 Catania, Italy
[h] International Joint Research Laboratory of Intelligent Agriculture and Agri-Products Processing, Jiangsu University, Zhenjiang, China

ARTICLE INFO

Keywords:
Aging population
Inflammation
Nutraceuticals
Oral health
Oxidative stress
Phytochemicals
Polyphenols

ABSTRACT

Background: Periodontitis is a prevalent chronic inflammatory disease in older adults, often linked to systemic conditions and metabolic alterations. Standardized plant extracts may provide consistent adjunctive therapeutic benefits.

Purpose and study design: This double-blind, randomized, placebo-controlled clinical trial (OLIVAGING; ClinicalTrials.gov: NCT05482373) evaluated whether a standardized olive leaf extract enriched to 40 % oleuropein, given in addition to non-surgical periodontal therapy, improves clinical outcomes and modulates systemic metabolism. Sixty participants aged ≥50 years were randomized to receive either supplement or placebo for 120 days. Primary and secondary outcomes were probing pocket depth (PPD) and clinical attachment level (CAL). Plasma metabolomics was performed using untargeted UHPLC-QTOF-MS.

Results: Forty-three participants completed the study (23 treatment, 20 placebo). Compared with placebo, the treatment group achieved greater reductions in ΔPPD and gains in ΔCAL across multiple tooth categories and surfaces. Metabolomic profiling revealed distinct Δ patterns, with 17 metabolites differing between groups. Several, including tentatively identified valine, cinnamic acid, 10-hydroxy-2-decenoic acid, and cortisol, correlated with periodontal improvements, suggesting modulation of biological pathways related to inflammation, oxidative stress, and tissue homeostasis consistent with the know pharmacological effects of oleuropein.

Conclusions: Adjunctive use of an oleuropein-enriched leaf extract improved clinical periodontal outcomes and modulated systemic metabolic signatures in older adults. The standardized extract ensured therapeutic consistency, while metabolomics provided mechanistic insights into host inflammatory and metabolic responses.

Abbreviations: 10-HAD, 10-hydroxy-2-decenoic acid; BCAA, Branched-Chain Amino Acids; CAL, Clinical Attachment Level; CONSORT, Consolidated Standards of Reporting Trials; EDTA, Ethylenediaminetetraacetic acid; EPA, Eicosapentaenoic Acid; IL-1β, Interleukin-1 beta; IL-8, Interleukin-8; LC, Liquid Chromatography; LC–HRMS, Liquid Chromatography–High Resolution Mass Spectrometry; MS/MS, Tandem Mass Spectrometry; NCT05482373, ClinicalTrials.gov Identifier of the OLIVAGING trial; NIST, National Institute of Standards and Technology; OLE, Olive Leaf Extract; PA, Phosphatidic Acid; PCA, Principal Component Analysis; PI3K/AKT/GSK3β pathway, Phosphatidylinositol-3-Kinase / Protein Kinase B / Glycogen Synthase Kinase 3 Beta signaling pathway; PLS-DA, Partial Least Squares Discriminant Analysis; PPD, Probing Pocket Depth; QC, Quality Control.

* Corresponding author at: Department of Physiology, Institute of Nutrition and Food Technology "José Mataix Verdú", Biomedical Research Centre, University of Granada, 18016 Armilla, Spain.
E-mail address: jlquiles@ugr.es (J.L. Quiles).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.phymed.2026.158222
Received 30 August 2025; Received in revised form 12 April 2026; Accepted 18 April 2026
Available online 19 April 2026

Findings support further evaluation of plant-derived bioactives as safe, multi-target adjunctive strategies for periodontitis management.

Introduction

Periodontitis is a highly prevalent chronic inflammatory disease that disproportionately affects older adults, with estimates suggesting that moderate-to-severe forms occur in up to 50–70 % of individuals over 50 years of age in Europe (Genco and Sanz, 2020). Beyond its oral manifestations, periodontitis is increasingly recognized as a component of a complex systemic inflammatory network and has been consistently associated with multiple systemic comorbidities, including cardiovascular disease, type 2 diabetes, metabolic syndrome, cognitive decline and others (Bullon et al., 2009). In this population, age-related impairments in tissue regenerative capacity, an altered inflammatory response, and shifts in the oral microbiota contribute to a reduced ability to recover from periodontal breakdown (Baima et al., 2022). Functionally, the disease compromises masticatory efficiency and nutritional status, and it has a significant impact on quality of life (Boehm and Scannapieco, 2007). Increasing evidence suggests that periodontitis should not only be viewed as a localized oral condition but rather as part of a broader network of systemic inflammatory and host–microbiota interactions (Hajishengallis and Chavakis, 2021). The oral cavity represents a major interface between the host and the external environment, and periodontal dysbiosis may contribute to systemic immune activation through multiple mechanisms, including dissemination of microbial products, chronic inflammatory signalling, and potential oral–gut microbial interactions. Emerging studies further support the concept of bidirectional relationships between periodontal disease and other inflammatory disorders, highlighting shared pathogenic pathways involving immune dysregulation, microbial imbalance, and metabolic alterations (Benameur et al., 2023; Genco and Sanz, 2020). Experimental and clinical evidence has also suggested that oral pathogens and inflammatory mediators may influence distant tissues, including the gastrointestinal tract, thereby reinforcing the systemic relevance of periodontal inflammation (Polizzi et al., 2025, 2024). These observations support the rationale for therapeutic strategies targeting host inflammatory and metabolic pathways beyond local periodontal treatment.

Mechanical debridement by scaling and root planning (SRP) remains the gold standard for non-surgical management of periodontitis. However, in older adults, SRP alone may be insufficient to fully resolve inflammation or to achieve optimal clinical attachment gain, particularly in advanced disease (Sanz et al., 2012). Persistent microbial dysbiosis and systemic low-grade inflammation can limit the long-term success of mechanical therapy (Moura et al., 2021). These limitations underscore the need for adjunctive strategies capable of modulating host inflammatory responses, supporting tissue repair, and potentially rebalancing the oral microbiome.

Natural bioactive compounds offer a promising approach in this context. Polyphenols from plant sources combine antioxidant, anti-inflammatory, and antimicrobial activities without the drawbacks of long-term antibiotic use, such as bacterial resistance or dysbiosis (Varela-López et al., 2015). Oleuropein, the main phenolic constituent of olive leaf extract (OLE), exhibits potent free radical scavenging capacity, inhibits key pro-inflammatory mediators (e.g., TNF-α, IL-1β), and suppresses the growth of major periodontal pathogens, including *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* (Karygianni et al., 2014).

In the present randomized controlled trial, we investigated the effects of an olive leaf extract enriched to 40 % oleuropein (OLE) as an adjunct to SRP in older adults with periodontitis. While multiple biological parameters were collected as part of the study (Forbes-Hernández et al., 2025), the present work focuses specifically on untargeted plasma metabolomics, given its unique potential to provide a comprehensive, system-wide view of host metabolic responses within the broader context of systemic inflammatory regulation. Clinical periodontal assessments were integrated with metabolomic profiling to explore whether adjunctive OLE supplementation could influence inflammatory- and oxidative stress–related pathways and how these metabolic alterations relate to changes in periodontal status. This targeted analytical approach was designed to generate mechanistic insights into the role of plant-derived bioactives as adjunctive strategies for improving periodontal health in this population. We hypothesized that adjunctive supplementation with oleuropein-enriched olive leaf extract would improve periodontal outcomes through complementary mechanisms involving anti-inflammatory and antioxidant effects, modulation of host–microbiota interactions, and systemic metabolic regulation contributing to tissue repair and periodontal homeostasis.

Methods

Study design and population

OLIVAGING is a double-blind, randomized, placebo-controlled study designed to evaluate the efficacy of a nutraceutical supplement derived from olive leaf extract, enriched to 40 % oleuropein (OLE, kindly provided by NATAC, Madrid, Spain), Fig. 1. This formulation, previously characterized by us (Romero-Márquez et al., 2022), was selected to ensure standardization of the bioactive compound, minimizing variability in phytochemical composition, and facilitating reproducibility and clinical translation. The same oleuropein-rich olive leaf extract has shown biological activity in previous experimental studies, supporting its selection for clinical evaluation (Romero-Márquez et al., 2022). Placebo capsules contained microcrystalline cellulose as inert excipient, whereas the active capsules contained the standardized olive leaf extract enriched to 40 % oleuropein together with microcrystalline cellulose. Both formulations were encapsulated in identical opaque white capsules of the same size and appearance. The study followed a double-blind design, whereby neither participants nor investigators involved in treatment administration or outcome assessment were aware of group allocation. Capsules were swallowed whole, minimizing the potential influence of taste on blinding. The study follows the CONSORT guidelines for randomized clinical trials and was registered at ClinicalTrials.gov (NCT05482373). A total of 60 participants aged ≥50 years with periodontitis were randomly assigned to either the treatment or placebo group in a 1:1 ratio. Over a 120-day intervention period, the treatment group received capsules containing 200 mg of OLE, enriched to 40 % oleuropein (equivalent to 80 mg oleuropein per day). On the other way, the control group received matching placebo capsules. Both groups underwent standard non-surgical periodontal therapy at baseline (Fig. 1). Participants were asked to take pills with food at the same hour of the day. Eligible participants were adults >50 years diagnosed with periodontitis, defined as interdental attachment loss in ≥2 non-adjacent teeth or buccal/lingual attachment loss ≥3 mm in ≥2 teeth. Exclusion criteria included antimicrobial therapy within the previous 6 months, anti-inflammatory drug use within the previous 2 months, regular vitamin supplementation, ongoing periodontal treatment, autoimmune diseases, systemic disorders affecting bone metabolism, aggressive periodontitis, or any condition affecting oral health or informed consent. A detailed description of the study design, inclusion and exclusion criteria, and baseline characteristics of the participants has been previously published by Forbes-Hernández et al (Forbes-Hernández et al., 2025). All procedures were approved by the Ethics Committee of the Andalusian Biomedical Research Ethics Portal (PEIBA code:

1588-N-20). Written informed consent was obtained from all participants prior to study enrolment.

Oral parameters

Non-surgical periodontal therapy (scaling and root planning) was performed at baseline by two trained periodontologists (PB and FGV-C) following a standardized clinical protocol. Both clinicians adhered to uniform treatment procedures to ensure consistency across participants. This intervention was performed immediately before the start of supplementation period. Clinical periodontal measurements, including probing pocket depth (PPD) and clinical attachment level (CAL), were assessed by the same experienced examiner (PB) using a manual periodontal probe (Hu-Friedy®, Chicago, IL, USA) at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual). For statistical analysis, measurements were aggregated by surface: the three buccal sites (mesiobuccal, buccal, distobuccal) were combined into a buccal surface value, and the three lingual sites (mesiolingual, lingual, distolingual) into a lingual surface value. For each participant, aggregated surface values were summed across teeth to obtain individual buccal and lingual totals used for statistical comparisons. Prior to study initiation, examiner calibration was performed in the first ten subjects to ensure reproducibility, yielding an intra-examiner correlation coefficient of 92 % for probing depth measurements. Full-mouth scaling and root planing, both manual and ultrasonic, were performed as part of the standard periodontal treatment. Participants also received ongoing oral hygiene instructions to support their treatment adherence. Changes in clinical parameters were expressed as ΔPPD and ΔCAL, calculated as post-intervention (T2) minus baseline (T1) values. These Δ values were used for all comparative and correlation analyses in the present study.

Untargeted metabolomic study

Metabolomic analyses were performed at Fundación MEDINA (Granada, Spain).

Materials and equipment

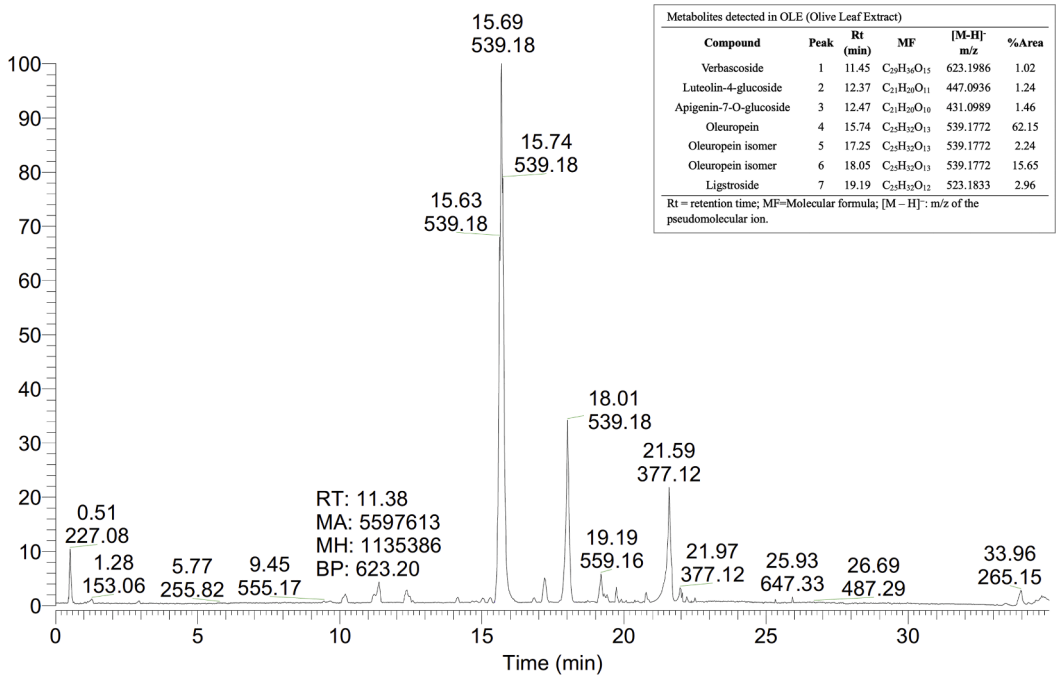
The analytical platform consisted of an Agilent 1290 Infinity UHPLC system (Agilent technologies, Santa Clara, CA, USA) coupled to a CTC Analytics PAL HT-xt autosampler (CTC Analytics AG, Zwingen, Switzerland) and a SCIEX TripleTOF® 5600 quadrupole time-of-flight mass spectrometer (SCIEX, Framingham, MA, USA). All solvents and reagents were of the highest available purity. Acetonitrile and methanol (HPLC grade; Merck, Darmstadt, Germany) were used for sample preparation and chromatographic separation. Internal standards included tryptophan-D5, chenodeoxycholic acid-D4, and cholic acid-C13 (Sigma-Aldrich, St. Louis, MO, USA).

Plasma collection and storage

Venous blood samples were collected into EDTA-containing tubes (BD Vacutainer®, Becton Dickinson, Franklin Lakes, NJ, USA) at baseline and at the end of the intervention. Samples were processed immediately after collection. Plasma was separated by centrifugation at 1500 × g for 10 min at 4 °C, aliquoted into cryovials to avoid repeated freeze–thaw cycles, and stored at –80 °C until metabolomic analysis. Changes in metabolite abundance were expressed as Δ metabolite (T2–T1), in parallel with the clinical variables.

Sample preparation

Plasma aliquots (100 µl) were mixed with 700 µl of cold acetonitrile



Metabolites detected in OLE (Olive Leaf Extract)

Compound	Peak	Rt (min)	MF	[M-H]⁻ m/z	%Area
Verbascoside	1	11.45	$C_{29}H_{36}O_{15}$	623.1986	1.02
Luteolin-4-glucoside	2	12.37	$C_{21}H_{20}O_{11}$	447.0936	1.24
Apigenin-7-O-glucoside	3	12.47	$C_{21}H_{20}O_{10}$	431.0989	1.46
Oleuropein	4	15.74	$C_{25}H_{32}O_{13}$	539.1772	62.15
Oleuropein isomer	5	17.25	$C_{25}H_{32}O_{13}$	539.1772	2.24
Oleuropein isomer	6	18.05	$C_{25}H_{32}O_{13}$	539.1772	15.65
Ligstroside	7	19.19	$C_{25}H_{32}O_{12}$	523.1833	2.96

Rt = retention time; MF=Molecular formula; $[M-H]^-$: m/z of the pseudomolecular ion.

Fig. 1. Total Ionic Chromatogram of OLE extract. The analyses were performed using an Accela High Speed LC System hyphenated to a hybrid LTQ Orbitrap XL discovery mass spectrometer (Thermo Scientific) equipped with an ESI source. An Ascentis Express Fused-Core™ C18 column (100 × 2.1 mm i.d., 2.7 µm, Supelco) was used for the separations. For the analysis of samples, the elution solvent system was (A) water 0.1 % formic acid and (B) acetonitrile, while the flow rate was set to 400 µl/min. The elution program was as follows: 5 % B for 2 min; 10 % B in 2.5 min; 20 % B in 13.5 min; 95 % B in 14 min and hold for 1 min; back to 5 % B in 0.5 min; column equilibration for 1.5 min. The extracts solutions were initially prepared in water–methanol 50:50 (v/v) and subjected to ultrasound and centrifugation process, at a final concentration of 400 µg/ml. The injection volume was 5 µl. The HRMS data were acquired in the negative mode over an *m/z* range of 100–1500. The MS profile was recorded in full scan mode (scan time = 1 micro scans and maximum inject time = 500 ms). The raw data was acquired and processed with Xcalibur 2.0.7 software from Thermo Scientific.

containing internal standards (100 ng/ml) to facilitate protein precipitation and metabolite extraction. Samples were vortex-mixed for 2 min at 1500 x g, followed by centrifugation at 16,000 × g for 15 min at 4 °C. Supernatants (700 µl) were collected and evaporated to dryness in a centrifugal evaporator (~2 h). Dried extracts were reconstituted in 150 µl of water/acetonitrile (50:50, v/v). Quality control (QC) samples were prepared by pooling 10 µl from each study sample; additional 1:10 and 1:100 QC dilutions were also prepared. A procedural blank was processed alongside the samples, and a NIST human plasma reference material (SRM 1950) was included as a system performance control.

Liquid chromatography and mass spectrometry analysis

Chromatographic separation was performed on an Agilent 1290 Infinity UHPLC coupled to a TripleTOF® 5600 mass spectrometer (SCIEX) with electrospray ionization in negative mode. Samples were injected onto an Atlantis® T3 C18 column (2.1 × 150 mm, 3 µm; Waters) at 30 °C. The mobile phase consisted of water and acetonitrile with 0.1 % formic acid, using a 20 min gradient from 1 % to 99 % B. Data were acquired in high-resolution TOF mode (*m/z* 50–1250) with information-dependent acquisition of the top eight ions. External calibration and pooled QC samples every 10 runs ensured analytical stability.

Data processing and analytical validation

Raw LC–HRMS data were processed in MarkerView™ (SCIEX) for peak detection, alignment, and filtering. Features were extracted within 1.5–16.0 min using a 12 ppm mass tolerance and 0.15 min retention tolerance. Monoisotopic peaks were retained, while background signals (fold change > 2 in blanks vs QC) and poorly reproducible features (RSD > 30 % in QC) were removed. PCA was applied to QC samples to assess analytical stability.

Identification of differential metabolites

Putative metabolite annotation used PeakView® (SCIEX) with accurate mass, isotopic patterns, and MS/MS fragmentation, complemented by Sirius (University of Jena). Spectral data were searched against METLIN, HMDB, LIPID MAPS, GNPS2, NIST 2021, and MassBank with a ± 5 ppm tolerance. Tentative identities were refined considering fragmentation patterns, ionization behaviour, retention time, and published data. Metabolite annotation followed Metabolomics Standards Initiative (MSI) criteria, with most annotated compounds corresponding to Level 2 (putatively annotated compounds), while some features were classified as Level 3 or Level 4.

Statistical analysis

Sample size calculation was based on the primary outcome (probing pocket depth, PPD), assuming a minimum clinically relevant detectable difference of 1 mm, a standard deviation of 0.5 mm, $\alpha = 0.05$ (two-tailed), and 80 % power ($\beta = 0.20$), with an anticipated attrition rate of 50 %, as previously detailed in the published trial protocol (Forbes-Hernández et al., 2025). The minimum required sample size was 32 participants (16 per group). Dental parameters were analysed with SPSS 24.0 (IBM, Armonk, NY, USA). Group comparisons were made at baseline, post-intervention, and for delta values (post–pre), which were the main outcomes. Normality (Kolmogorov–Smirnov) and homogeneity of variance (Levene's test) were verified; outliers (> mean ± 3 SD) were excluded. Between-group differences were assessed with independent Student's *t*-test ($p < 0.05$). Metabolomic data were processed and analysed using MetaboAnalyst 6.0 (Pang et al., 2024). After log transformation and Pareto scaling, univariate analysis (*t*-test with fold change) was visualized in volcano plots. Multivariate analysis by PLS-DA assessed group separation. Pearson correlation coefficients were calculated to evaluate associations between changes in metabolite abundance (ΔT2–T1) and changes in clinical parameters (ΔPPD and ΔCAL) using the MetaboAnalyst platform. Correlation analyses were exploratory in nature and were not adjusted for additional covariates due to the limited sample size.

Results

Of the 60 randomized participants, 43 completed the 120-day intervention and were included in the final analysis, 23 in the OLE group and 20 in the placebo group (Fig. 2). Although discontinuations were slightly higher in the placebo group, no withdrawals were related to adverse events. Baseline demographic and clinical characteristics were comparable between groups. No statistically significant differences were observed between the placebo and OLE groups in age (61.95 ± 6.35 *vs.* 61.40 ± 11.31 years), body weight (77.5 ± 15.8 *vs.* 74.4 ± 10.2 kg), or height (1.69 ± 0.09 *vs.* 1.68 ± 0.08 m). Similarly, baseline periodontal parameters showed no significant intergroup differences. For example, total probing pocket depth (PPD) at the whole-mouth level was 70.25 ± 22.23 mm vs. 71.31 ± 27.69 mm (buccal) and 65.30 ± 18.08 mm vs. 72.79 ± 30.28 mm (lingual), while total clinical attachment level (CAL) values were 80.15 ± 21.04 mm vs. 95.75 ± 38.87 mm (buccal) and 80.53 ± 19.31 mm vs. 97.29 ± 41.87 mm (lingual), in placebo and OLE groups respectively. Additional baseline characteristics are detailed in the previously published trial protocol (Forbes-Hernández et al., 2025). Table 1 presents within-group absolute mean changes (ΔT2–T1 ± SD) for each clinical parameter. At 120 days, both groups showed reductions in probing pocket depth (ΔPPD), but decreases were consistently greater with olive leaf extract. Significant intergroup differences appeared in maxillary incisors, canines, and premolars on buccal surfaces, and in incisors and canines on lingual surfaces. In the mandible, differences were observed for premolars (buccal) and incisors and molars (lingual). When pooled, the treatment group showed significantly higher total ΔPPD on both buccal (–14.90 ± 14.14 mm vs. –8.28 ± 5.13 mm; $p < 0.05$) and lingual surfaces (–14.47 ± 15.59 mm vs. –8.27 ± 5.87 mm; $p < 0.05$). Clinical attachment level (ΔCAL) improved in both groups (Table 1). Gains were greater with treatment, particularly for lingual canines and premolars, but only reached significance for canines at the pooled whole-mouth level. Safety monitoring revealed no serious adverse events in either group.

Untargeted metabolomics detected 264 metabolites. At baseline, PLS-DA (Fig. 3A) and volcano plots (Fig. 4A) showed no group differences, confirming metabolic homogeneity. In contrast, Δ abundance analysis revealed clear separation post-intervention (Fig. 4B), with 17 metabolites differing significantly between groups. Detailed univariate results, including tentative identification, *m/z* values, retention times, group means ± SD, fold change (ΔTreatment/ΔControl), raw p-values (two-tailed Welch's *t*-test), FDR-adjusted p-values (Benjamini–Hochberg), and PLS-DA VIP scores, are provided in Supplementary Table S1. Several metabolites remained significant after FDR correction ($q < 0.10$), supporting the robustness of the observed metabolic modulation. Boxplots (Figs. 5 and 6) confirmed these patterns. Tentative identifications followed Schymanski classification (Schymanski et al., 2014); not all metabolites could be assigned. VIP analysis (Fig. 7) highlighted ursodeoxycholic acid, cortisol, 10-hydroxy-2-decenoic acid, valine, cinnamic acid, biliverdin, eicosapentaenoic acid, and several unidentified metabolites as key contributors to group discrimination (VIP >1). Correlation analysis (Table 2; Figs. 8 and 9) linked ΔPPD improvements with higher valine, cinnamic acid, 10-hydroxy-2-decenoic acid, and MTB174 (negative correlations), while ΔCAL gains were associated with MTB218, MTB232, and MTB32. Some metabolites showed divergent associations with ΔPPD and ΔCAL, suggesting distinct pathways underlying pocket depth reduction and attachment gain. The complete correlation matrices, including Pearson coefficients and corresponding p-values for all tested metabolites, are provided in the Supplementary Material (Figures S1–S2; Tables S2–S5).

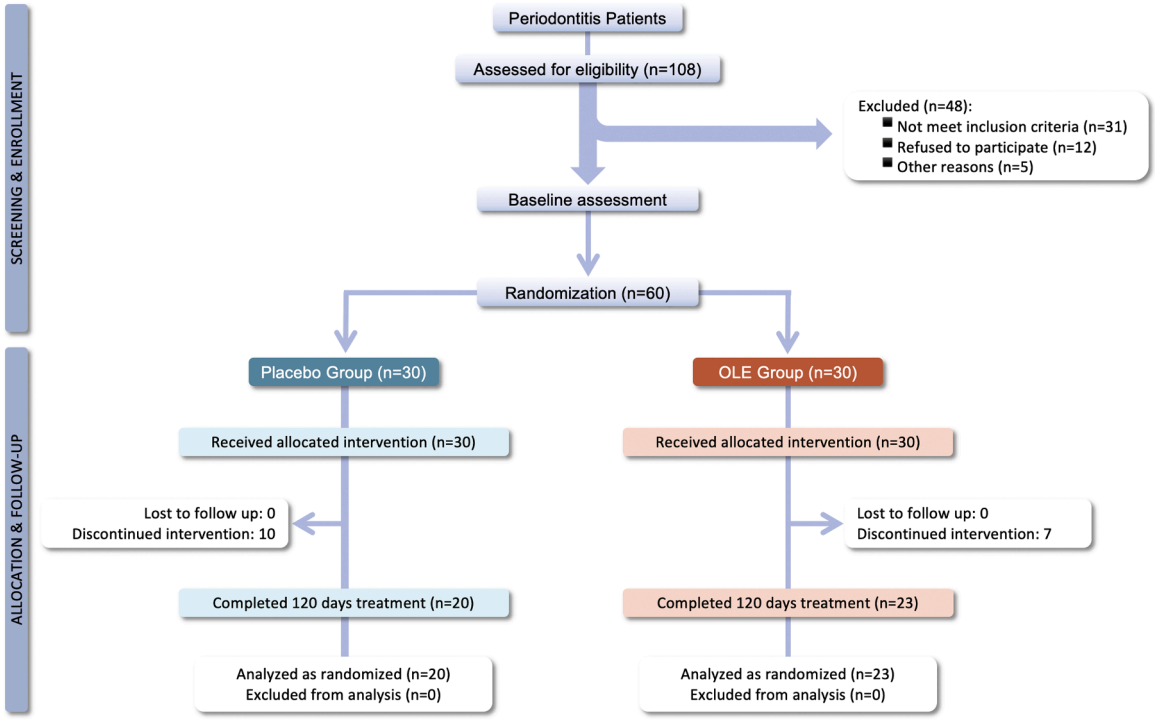


Fig. 2. CONSORT (consolidated standards of reporting trials) statement of the study.

Discussion

Previous clinical studies in older patients have shown that conventional non-surgical periodontal therapy, while effective, often yields less durable benefits than in younger populations, largely due to reduced healing, comorbidities, and higher baseline inflammation (Moura et al., 2021; Sanz et al., 2012). Adjunctive strategies such as systemic antibiotics (Hammami and Nasri, 2021), local antimicrobials (Herrera et al., 2020), and photodynamic therapy (Alves et al., 2022), have been tested, but their long-term efficacy and safety are uncertain. To our knowledge, this is the first randomized, double-blind, placebo-controlled clinical trial evaluating a standardized oleuropein-enriched olive leaf extract as an adjunct to non-surgical periodontal therapy in older adults, integrating clinical periodontal outcomes with systemic untargeted metabolomics. In this trial, olive leaf extract enriched in oleuropein produced greater reductions in ΔPPD and gains in ΔCAL than standard therapy. Although ΔCAL improvements were modest and not significant at the whole-mouth level, the overall pattern suggests enhanced healing. These findings support adjunctive approaches in older adults with diminished regenerative capacity and persistent inflammation (Moura et al., 2021; Sanz et al., 2012). Previous trials with antibiotics, photodynamic therapy, or locally delivered antimicrobials have reported additional PPD reductions typically ranging from approximately 0.2 to 0.6 mm beyond scaling and root planning alone, depending on baseline severity and study design (Kondreddy et al., 2025; Teughels et al., 2020). Host-modulatory approaches, such as sub-antimicrobial-dose doxycycline, have also demonstrated modest adjunctive gains in clinical attachment and pocket depth (Preshaw et al., 2004). While some systemic antimicrobial strategies may yield slightly greater short-term improvements, they are frequently associated with safety concerns and the risk of antimicrobial resistance. In contrast, the present results indicate that a phytochemical-based adjunct can achieve meaningful improvements without antimicrobial drugs, suggesting a more sustainable strategy. The 120-day follow-up corresponds to the standard 3–4 month periodontal re-evaluation interval after non-surgical therapy (Sanz et al., 2012), allowing resolution of inflammation and stabilization of probing depth and attachment levels. Although longer follow-up periods would be required to assess long-term structural changes, this time frame is considered appropriate for evaluating early clinical response to adjunctive treatment.

Numerous recent reviews support that plant-derived bioactive compounds—such as polyphenols, flavonoids, and other phytochemicals—act as multi-target modulators of inflammation, oxidative stress, and microbial dysbiosis, without the drawbacks associated with antimicrobial drugs. For instance, natural bioactive compounds can preserve gut microbiota while offering antioxidant and anti-inflammatory effects (Benameur et al., 2023). In addition, comprehensive analyses of immunomodulatory plants, including *Olea europaea*, highlight their safe and effective action on multiple pathological pathways (Vieira et al., 2025). Olive leaf extracts, particularly when enriched in oleuropein, has been reported to exhibit potent antioxidant and anti-inflammatory effects, as well as antimicrobial activity against *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans*, and other periodontopathogens (Karygianni et al., 2014). However, to our knowledge, no previous randomized controlled trial has examined its use as an adjunct to standard non-surgical periodontal therapy in older adults. Beyond periodontitis, olive leaf extracts and its principal phenolic constituent oleuropein have been studied in various inflammatory and degenerative conditions, many of which share mechanistic pathways with periodontal disease (Romero-Márquez et al., 2022). Other studies have reported protective effects of OLE supplementation in metabolic syndrome, cardiovascular risk, and exercise-induced oxidative stress, further supporting its systemic bioactivity (Lillis et al., 2025). Collectively, these findings suggest that OLE could exert beneficial effects on periodontal health not only via local antimicrobial and anti-inflammatory actions but also through systemic modulation of host inflammatory and metabolic responses. It should not be overlooked that oxidative stress plays a central role in the pathogenesis and progression of periodontitis, contributing to connective tissue breakdown and alveolar bone loss through the excessive production of reactive oxygen species and subsequent amplification of inflammatory cascades (Bullon et al., 2025). The biological effects observed in this study are consistent with the known pharmacological properties of oleuropein, which is considered to exert a multifactorial mechanism of action relevant to periodontal

Table 1
Mean absolute changes (ΔT2–T1) ± SD in Probing Pocket Depth (PPD) and Clinical Attachment Loss (CAL) by tooth type and surface in periodontitis patients treated (n = 23) or not (n = 20) with the olive leaf enriched extract (OLE) in oleuropein for 120 days.

	Probing Pocket Depth (PPD)												Clinical Attachment Loss (CAL)											
	Maxillary												Maxillary											
	Buccal						Lingual						Buccal						Lingual					
	Control			Treatment			Control			Treatment			Control			Treatment			Control			Treatment		
Incisors	−1,15	±	1,47	−2,50	±	3,03*	−1,33	±	1,64	−2,54	±	3,41*	−0,90	±	1,41	−1,20	±	2,46	−0,84	±	1,93	−1,51	±	2,65
Canines	−0,54	±	0,95	−1,35	±	1,43*	−0,40	±	0,57	−1,39	±	1,68*	−0,30	±	0,98	−0,39	±	1,41	−0,06	±	1,33	−0,62	±	1,35*
Premolars	−0,75	±	1,42	−1,88	±	2,39*	−0,73	±	1,49	−1,58	±	2,54	−0,98	±	1,98	−0,83	±	1,78	−0,28	±	1,96	−1,15	±	2,14*
Molars	−1,28	±	1,40	−1,68	±	2,90	−2,29	±	2,28	−1,51	±	3,01	−1,00	±	1,79	−1,18	±	3,69	−0,83	±	1,98	−1,00	±	3,81
Total	−4,13	±	3,92	−7,40	±	7,60*	−4,72	±	4,26	−7,03	±	8,00	−4,13	±	5,00	−4,69	±	8,40	−2,92	±	5,90	−5,32	±	8,58
	Mandible												Mandible											
	Buccal						Lingual						Buccal						Lingual					
	Control			Treatment			Control			Treatment			Control			Treatment			Control			Treatment		
Incisors	−2,17	±	1,62	−2,50	±	4,08	−1,29	±	1,67	−2,58	±	3,94*	−1,67	±	2,69	−0,65	±	1,79	−2,17	±	2,14	−2,62	±	5,12
Canines	−0,79	±	0,88	−1,00	±	1,71	−0,73	±	0,83	−0,82	±	1,74	−0,46	±	1,03	−0,39	±	1,43	−0,65	±	1,79	−0,17	±	1,46
Premolars	−0,87	±	1,80	−2,19	±	2,30*	−1,00	±	1,51	−2,06	±	3,03	−0,29	±	2,13	−0,97	±	2,40	−0,49	±	1,32	−1,07	±	2,51
Molars	−0,62	±	1,30	−1,85	±	3,59	−0,62	±	1,46	−1,99	±	3,74*	−0,62	±	1,32	−1,51	±	4,18	−0,57	±	2,28	−1,50	±	3,78
Total	−4,15	±	3,11	−7,54	±	8,34*	−3,18	±	2,30	−7,44	±	9,01*	−3,41	±	4,09	−4,19	±	7,37	−3,89	±	4,82	−5,17	±	7,99
	Total mouth												Total mouth											
	Buccal						Lingual						Buccal						Lingual					
	Control			Treatment			Control			Treatment			Control			Treatment			Control			Treatment		
Incisors	−3,70	±	3,06	−5,00	±	6,42*	−2,95	±	3,22	−5,13	±	6,55*	−2,57	±	2,65	−2,78	±	5,73	−3,02	±	2,92	−4,13	±	6,02
Canines	−1,10	±	1,08	−2,35	±	2,71*	−1,30	±	1,55	−2,21	±	2,71*	−0,76	±	1,25	−1,49	±	3,11	−0,27	±	1,82	−1,44	±	2,26*
Premolars	−2,10	±	2,30	−4,07	±	3,98*	−2,22	±	2,30	−3,64	±	4,82	−1,81	±	2,90	−2,28	±	4,03	−0,75	±	2,57	−2,22	±	3,68
Molars	−1,90	±	1,97	−3,54	±	4,84	−2,58	±	2,12	−3,50	±	5,25	−1,62	±	2,27	−2,69	±	5,61	−1,12	±	2,39	−2,50	±	6,05
Total	−8,28	±	5,13	−14,90	±	14,14*	−8,27	±	5,87	−14,47	±	15,59*	−7,54	±	6,50	−7,86	±	10,16	−6,81	±	8,99	−9,46	±	11,98

Results represents mean ± SD of the changes between post-intervention (T2) and pre-intervention (T1). D Negative values indicate a reduction in PPD or CAL.
* Statistically significant different between control and treatment group ($p < 0.05$).

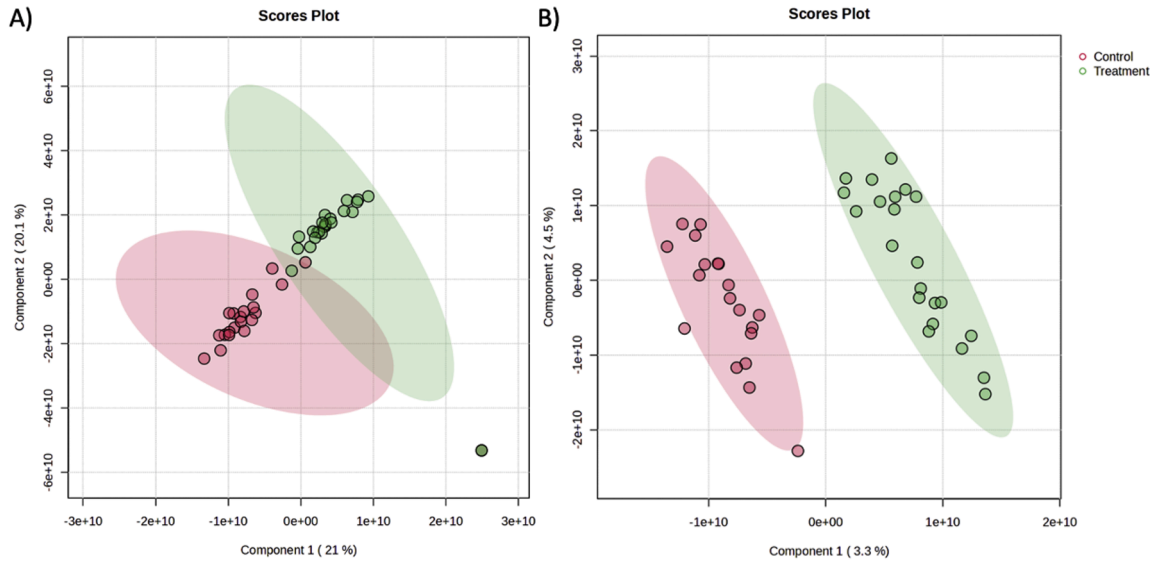


Fig. 3. Partial Least Square Discriminant Analysis (PLSDA). A) Control vs. treatment groups at T1 (the start of the study). B) Control vs. treatment group at T2 – T1 (D). No separation was observed at baseline, confirming metabolic homogeneity, whereas post-intervention Δ analysis demonstrated clear discrimination between control and treatment groups. Ellipses represent 95 % confidence intervals.

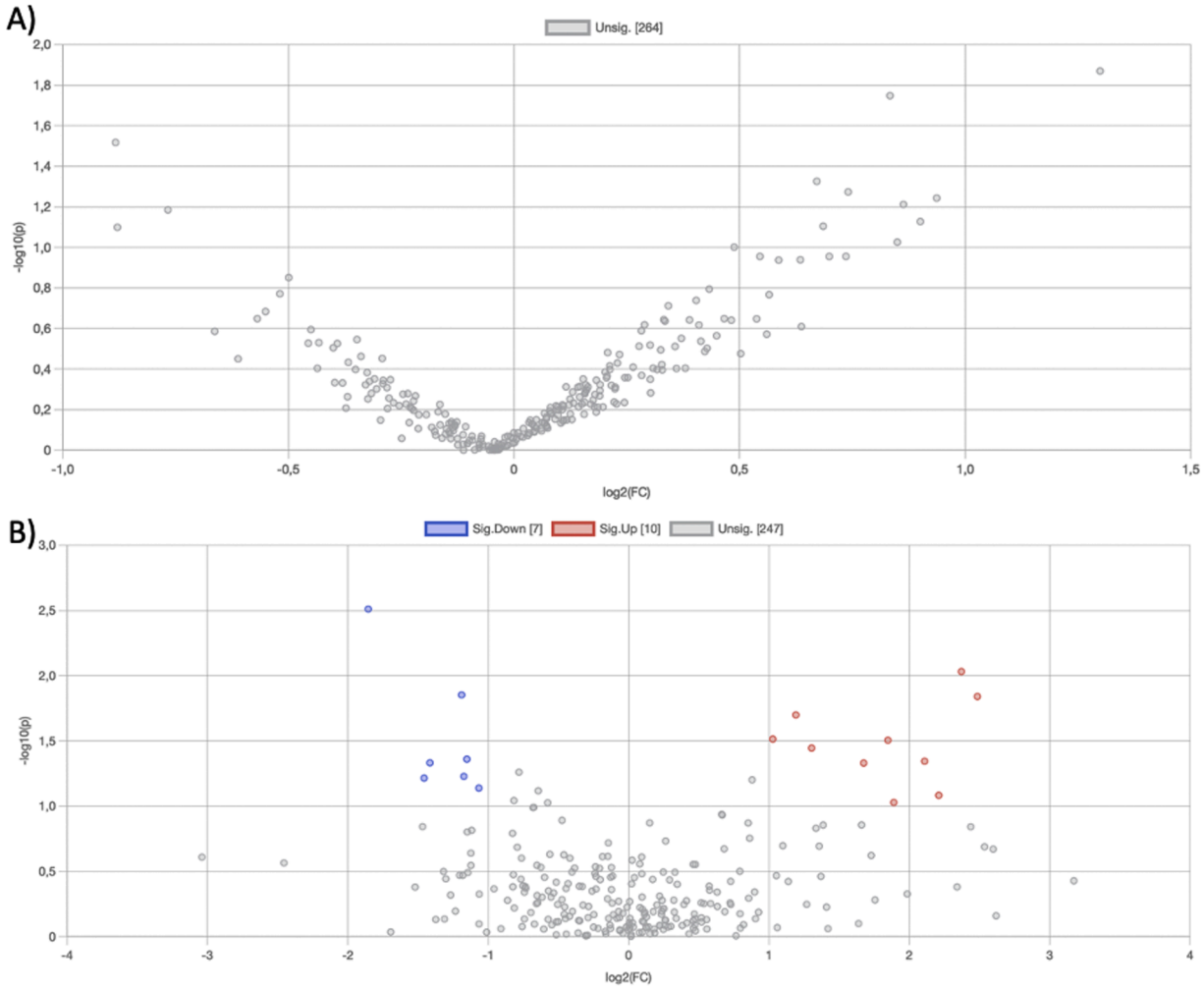


Fig. 4. Volcano plot. A) Control vs. treatment groups at T1 (the start of the study). B) Control vs. treatment group at T2 – T1 (D).

disease pathophysiology. Oleuropein has been reported to modulate inflammatory responses by inhibiting pro-inflammatory mediators,

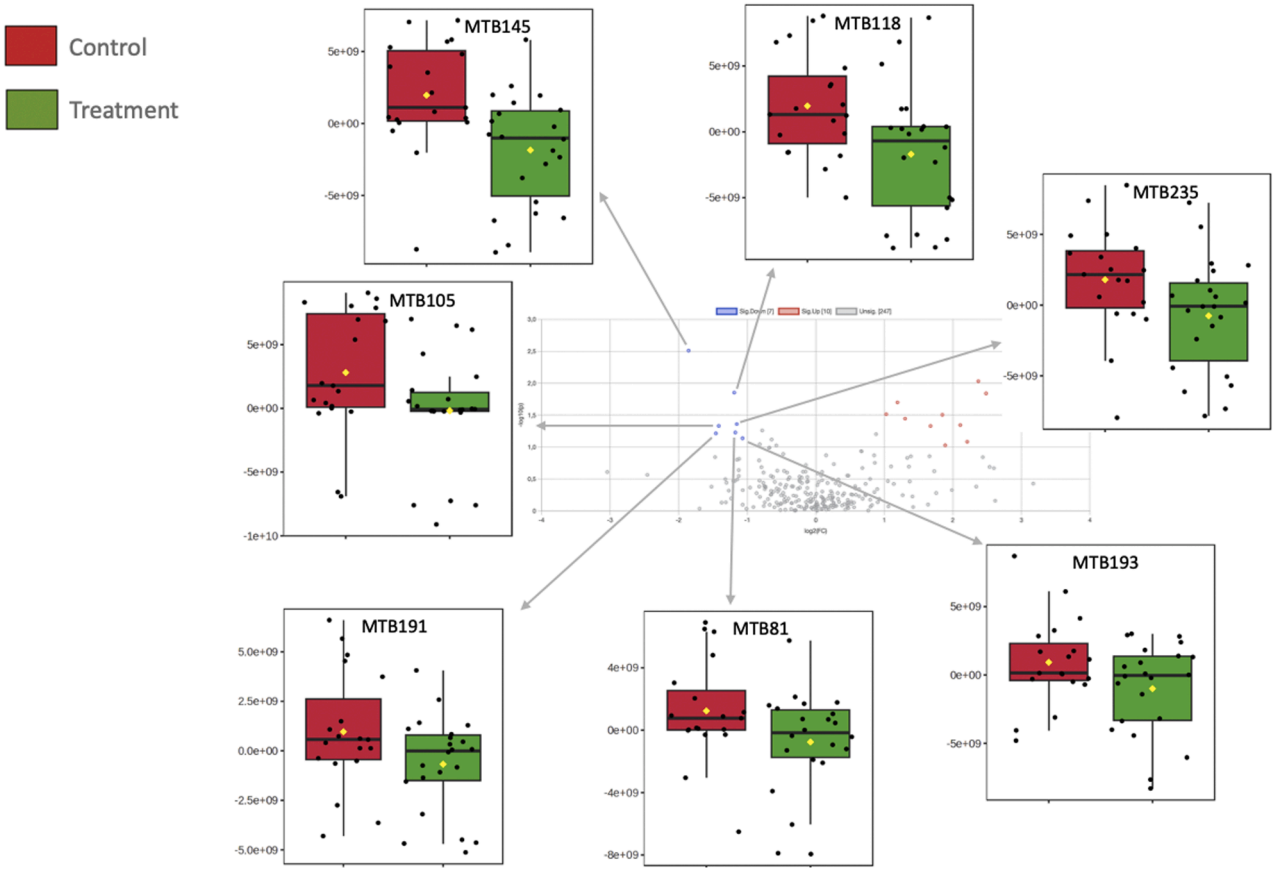


Fig. 5. Volcano plot of Control vs. treatment group at T2 – T1 (D) featuring boxplot for metabolites (MTB) significantly underrepresented in the treatment group compared to control group.

reduce oxidative stress through its antioxidant activity, and exert antimicrobial effects against key periodontal pathogens. In addition, emerging evidence suggests that olive-derived polyphenols may influence host metabolic and immune pathways involved in tissue repair and homeostasis. These complementary mechanisms may collectively contribute to the modulation of periodontal inflammation and support the systemic metabolic adaptations observed in the present study (Omar, 2010).

A strength of this intervention is the use of a standardized enriched extract. Natural plant products often show variability in phytochemical composition due to cultivar, origin, season, processing, and storage (Ogwu et al., 2025), which limits reproducibility and dose–response assessment. By enriching the extract to 40 % oleuropein, this preparation ensures greater therapeutic consistency, improved reproducibility, reduced non-active components, and preserved acceptability—key attributes for clinical translation.

Metabolomics has emerged as a powerful system-level tool in biomedical research, enabling the comprehensive characterization of small-molecule metabolites that reflect both endogenous physiological processes and exogenous exposures, including diet and nutraceutical supplementation (Gonzalez-Covarrubias et al., 2022). Because metabolites are the end products of complex networks, their profiles integrate information from genomics, proteomics, microbiome activity, and environment, providing a direct readout of biochemical state. This is highly relevant for plant-derived bioactives, which act pleiotropically on multiple pathways. Metabolomics thus enables linking supplementation to mechanisms and identifying biomarkers of efficacy (Shah and Newgard, 2015). In the present trial, the analysis of Δ metabolite abundance (T2–T1) revealed distinct systemic metabolic shifts in participants receiving the olive leaf extract compared with controls. Notably, several of these metabolites were tentatively identified and correlated with ΔPPD and ΔCAL, suggesting that the clinical benefits of OLE may be mediated, at least in part, through modulation of host metabolic pathways related to inflammation, oxidative stress, and tissue homeostasis.

Several of the metabolites tentatively identified as differing between groups and correlating with ΔPPD or ΔCAL are consistent with known pathways implicated in periodontal disease pathophysiology. For example, valine (MTB05), a branched-chain amino acid, was more increased in the treatment group and negatively correlated with ΔPPD, suggesting a potential link with improved protein metabolism and tissue repair processes. Branched-chain amino acids have been associated with modulating muscle and connective tissue metabolism, and their elevation here may reflect enhanced anabolic or regenerative capacity during periodontal healing (Spahr and Divnic-Resnik, 2022). Although elevated branched-chain amino acids have been linked to metabolic syndrome in chronic systemic conditions, their biological interpretation is context-dependent. Branched-chain amino acids such as valine not only serve as essential building blocks for protein synthesis, but also function as signalling molecules involved in immune cell metabolism and metabolic reprogramming during inflammatory resolution and tissue repair, with documented roles in lymphocyte proliferation and immune regulation (Yahsi and Gunaydin, 2022).

Cinnamic acid (MTB17), a phenylpropanoid with known antioxidant and anti-inflammatory properties (Ruwizhi and Aderibigbe, 2020), also showed greater increases in the treatment group and favourable correlations with periodontal improvement. Although cinnamic acid derivatives have demonstrated antimicrobial activity in vitro, particularly against certain oral pathogens, the systemic administration route in the present study makes a direct bactericidal effect within the periodontal pocket less certain. Cinnamic acid can be derived from dietary sources

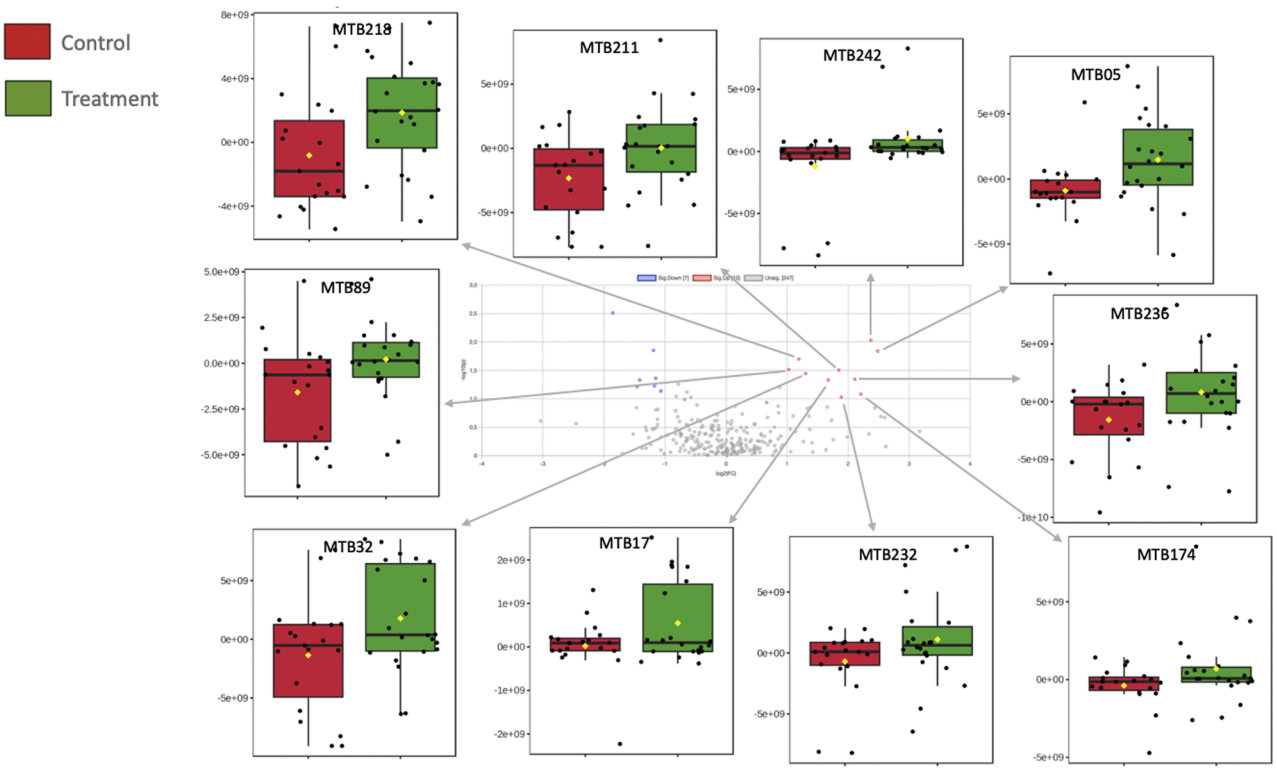


Fig. 6. Volcano plot of Control vs. treatment group at T2 – T1 (D) featuring boxplot for metabolites (MTB) significantly overrepresented in the treatment group compared to control group.

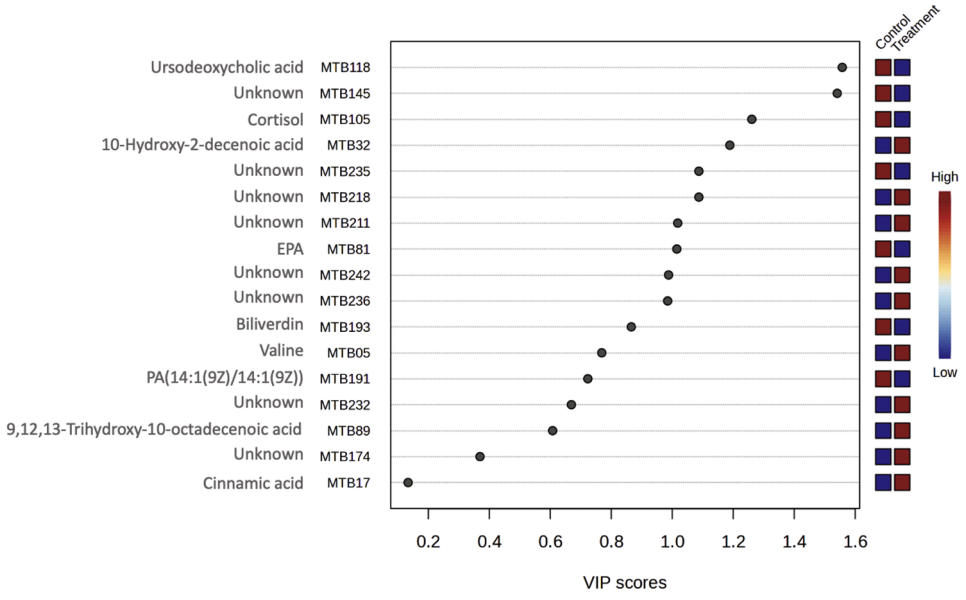


Fig. 7. Variable importance in projection (VIP) analysis performed on the 17 metabolites differing significantly between control and treatment groups.

but may also arise from microbial metabolism of polyphenols, suggesting that OLE supplementation may influence host–microbiota metabolic interactions rather than exerting a purely antimicrobial effect. In this context, the observed association may reflect modulation of inflammatory and metabolic pathways linked to periodontal healing.

Biliverdin (MTB193), and related bile pigment metabolites, are potent endogenous antioxidants produced via heme oxygenase activity (Gonzalez-Sanchez et al., 2016). Although biliverdin levels were higher in controls, their negative correlations with periodontal parameters suggest a compensatory up-regulation in response to greater oxidative stress in the absence of OLE supplementation. This is consistent with the hypothesis that OLE may reduce oxidative stress burden, diminishing the need for endogenous antioxidant up-regulation.

Cortisol (MTB105), a glucocorticoid hormone linked to stress and systemic inflammation (Morey et al., 2015), was more elevated in controls and negatively correlated with ΔPPD and ΔCAL, suggesting that greater cortisol increases were associated with poorer periodontal improvement. Lower cortisol increases in the treatment group may

Table 2
Correlations between changes in periodontal clinical parameters (total-mouth buccal PPD, ΔPPD, and total-mouth buccal CAL, ΔCAL, both T2–T1) and changes in relative abundance of selected plasma metabolites (T2–T1) in periodontitis patients treated (n = 23) or not (n = 20) with olive leaf extract enriched in oleuropein (OLE) for 120 days.

Metabolite (tentative identification)	Higher in…	Correlation with ΔPPD buccal	Interpretation
MTB05 (Valine)	Treatment	Negative	Higher post-intervention valine levels associated with greater PPD reduction (consistent pattern).
MTB17 (Cinnamic acid)	Treatment	Negative	Higher cinnamic acid linked to greater PPD reduction (consistent).
MTB32 (10-Hydroxy-2-decenoic acid)	Treatment	Negative	Increase associated with greater clinical improvement (consistent).
MTB81 (EPA)	Control	Positive	Higher EPA associated with smaller PPD reduction; lower levels in treatment group align with improvement.
MTB89 (9,12,13-Trihydroxy-10-octadecenoic acid)	Control	Positive	Higher levels linked to smaller improvement; reduction in treatment group consistent with improvement.
MTB105 (Cortisol)	Control	Negative	Higher cortisol linked to greater PPD reduction, but treatment group has lower levels (inverse pattern).
MTB118 (Ursodeoxycholic acid)	Control	Negative	Higher levels linked to greater PPD reduction; lower levels in treatment group suggest inverse relationship.
MTB145 (Unknown)	Control	Positive	Higher levels linked to smaller improvement; lower in treatment group consistent with improvement.
MTB174 (Unknown)	Treatment	Negative	Increase associated with greater PPD reduction (consistent).
MTB191 (Phosphatidic acid [14:1(9Z)/14:1 (9Z)])	Control	Negative	Higher levels linked to greater PPD reduction; lower in treatment group suggests inverse relationship.
MTB193 (Biliverdin)	Control	Negative	Higher levels linked to greater PPD reduction; lower in treatment group suggests inverse relationship.
MTB211 (Unknown)	Control	Near zero	Minimal correlation; limited interpretation.
MTB218 (Unknown)	Treatment	Positive	Increase associated with smaller improvement (inverse/unfavourable pattern).
MTB232 (Unknown)	Treatment	Positive	Increase associated with smaller improvement (inverse/unfavourable pattern).
MTB235 (Unknown)	Control	Near zero	Minimal correlation; limited interpretation.
MTB236 (Unknown)	Treatment	Positive	Increase associated with smaller improvement (inverse/unfavourable pattern).
MTB242 (Unknown)	Treatment	Positive	Increase associated with smaller improvement (inverse/unfavourable pattern).
Metabolite (tentative identification)	Higher in…	Correlation with ΔCAL buccal	Interpretation
MTB236 (Unknown)	Treatment	Positive	Increase associated with smaller CAL gain (inverse/unfavourable pattern).
MTB17 (Cinnamic acid)	Treatment	Positive	Increase associated with smaller CAL gain (inverse/unfavourable pattern).
MTB05 (Valine)	Treatment	Positive	Increase associated with smaller CAL gain (inverse/unfavourable pattern).
MTB81 (EPA)	Control	Positive	Higher levels linked to smaller CAL gain; lower in treatment group consistent with improvement.
MTB242 (Unknown)	Treatment	Positive	Increase associated with smaller CAL gain (inverse/unfavourable pattern).
MTB193 (Biliverdin)	Control	Near zero	Minimal correlation; limited interpretation.
MTB174 (Unknown)	Treatment	Near zero	Minimal correlation; limited interpretation.
MTB145 (Unknown)	Control	Near zero	Minimal correlation; limited interpretation.
MTB191 (Phosphatidic acid [14:1(9Z)/14:1 (9Z)])	Control	Negative	Higher levels linked to greater CAL gain; lower in treatment group suggests inverse relationship.
MTB118 (Ursodeoxycholic acid)	Control	Negative	Higher levels linked to greater CAL gain; lower in treatment group suggests inverse relationship.
MTB235 (Unknown)	Control	Negative	Higher levels linked to greater CAL gain; lower in treatment group suggests inverse relationship.
MTB211 (Unknown)	Control	Negative	Higher levels linked to greater CAL gain; lower in treatment group suggests inverse relationship.
MTB105 (Cortisol)	Control	Negative	Higher levels linked to greater CAL gain; lower in treatment group suggests inverse relationship.
MTB218 (Unknown)	Treatment	Negative	Increase associated with greater CAL gain (consistent).
MTB32 (10-Hydroxy-2-decenoic acid)	Treatment	Negative	Increase associated with greater CAL gain (consistent).
MTB89 (9,12,13-Trihydroxy-10-octadecenoic acid)	Control	Negative	Higher levels linked to greater CAL gain; lower in treatment group suggests inverse relationship.
MTB232 (Unknown)	Treatment	Negative	Increase associated with greater CAL gain (consistent).

Notes: ΔPPD and ΔCAL represent the change between post-intervention (T2) and pre-intervention (T1) in probing pocket depth and clinical attachment level, respectively. Metabolite changes are expressed as differences in relative

abundance between T2 and T1 within each group. "↑ Treatment" or "↑ Control" indicate higher net change in the respective group. Pearson's correlation coefficients (R) describe the association between metabolite changes and ΔPPD or ΔCAL; negative correlations indicate that greater metabolite increases were associated with greater clinical improvement, whereas positive correlations indicate the opposite.

reflect attenuation of stress-related inflammatory activation. Although oleuropein is not classically described as a direct hypothalamic–pituitary–adrenal (HPA) axis modulator, experimental studies have reported neuroprotective and anti-inflammatory effects of olive polyphenols, including modulation of oxidative stress and cytokine signalling pathways that can influence neuroendocrine stress responses. Therefore, the observed cortisol pattern may represent an indirect consequence of reduced systemic inflammatory burden rather than a primary endocrine effect. Further targeted studies would be required to determine whether olive phenolics directly influence HPA-axis regulation in humans.

Several unidentified lipid species, including phosphatidic acid PA (14:1(9Z)/14:1(9Z)) (MTB191) and other long-chain fatty acid derivatives showed patterns suggestive of altered membrane lipid

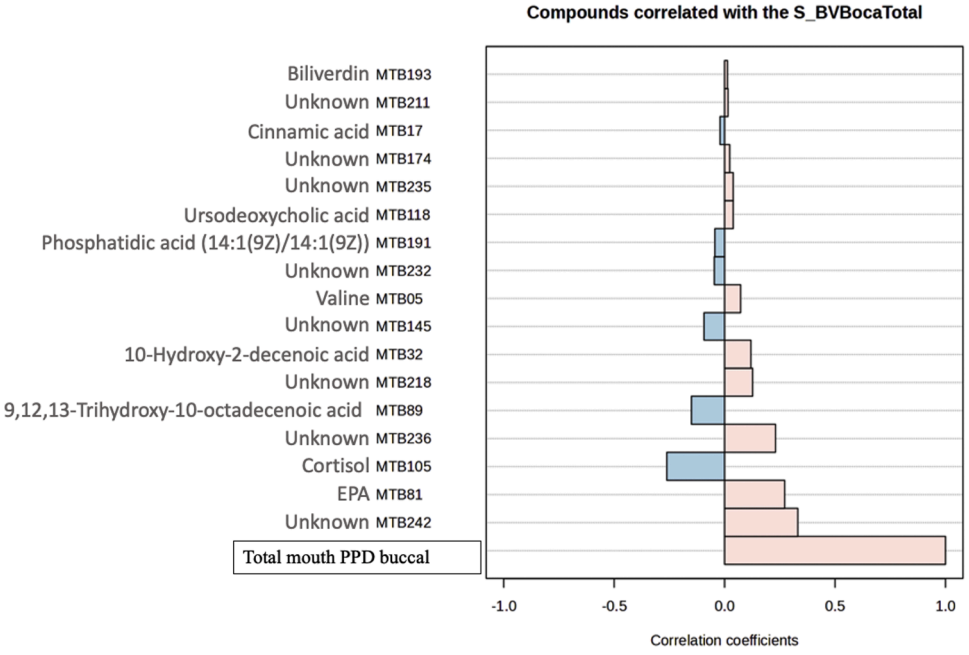


Fig. 8. Correlation pattern (Pearson's R) between total-mouth buccal PPD (ΔPPD) and the 17 metabolites significantly different between groups.

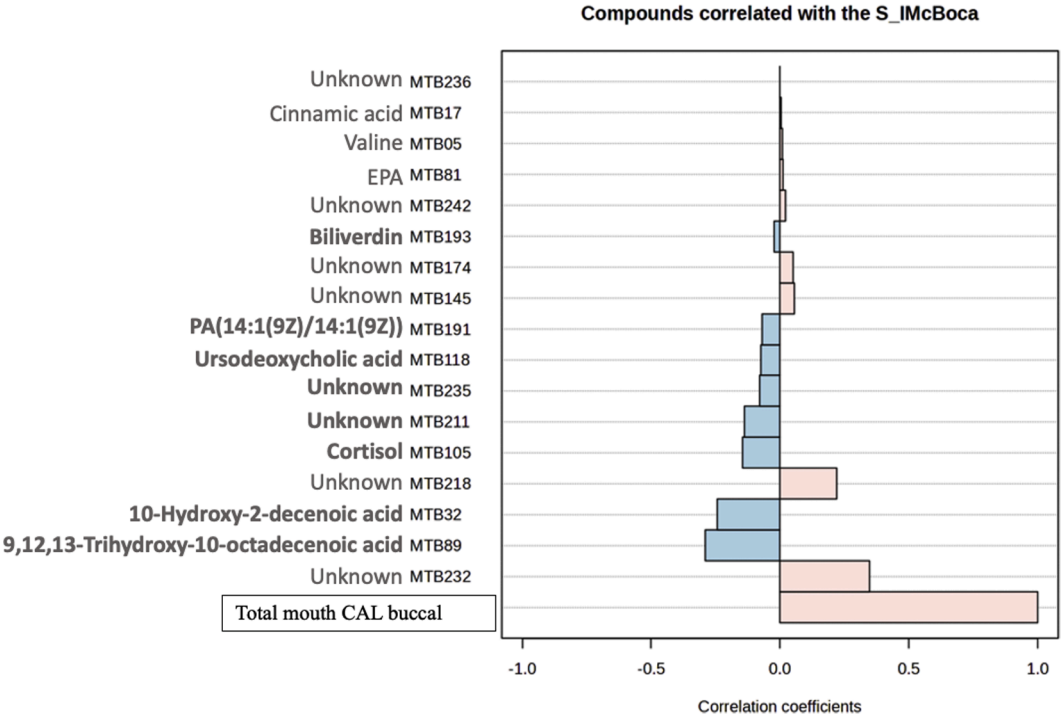


Fig. 9. Correlation pattern (Pearson's R) between total-mouth buccal CAL (ΔCAL) and the 17 metabolites significantly different between groups.

metabolism and inflammatory signalling. Such changes could be linked to the modulation of eicosanoid biosynthesis or membrane remodelling processes, both of which are relevant to periodontal inflammation and repair (Biernacki and Skrzydlewska, 2025).

In our study, 10-hydroxy-2-decenoic acid (10-HDA, MTB32) was significantly elevated in the treatment group and exhibited consistent negative correlations with both ΔPPD and ΔCAL, indicating better periodontal outcomes in individuals with higher post-intervention levels. This unique medium-chain fatty acid, classically described as a major component of royal jelly, has demonstrated anti-inflammatory, antibacterial, and cytoprotective effects in various in vitro and in vivo models, including attenuation of lipopolysaccharide-induced nitric oxide production and suppression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-8 (Chen et al., 2016). Beyond its antimicrobial and immunomodulatory activities, 10-HDA has also been shown to improve glucose metabolism and insulin sensitivity via activation of the PI3K/AKT/GSK3β pathway in diabetic mice (Hu et al., 2022), suggesting broader systemic metabolic benefits that could support tissue repair processes. Importantly, 10-HDA is not a known direct metabolite of oleuropein. Therefore, its elevation in the treatment group more likely reflects broader modulation of host lipid metabolism or microbial-associated lipid pathways rather than a direct metabolic conversion of olive phenolics. Oleuropein has been shown to influence oxidative stress responses and lipid homeostasis; thus, the observed increase in 10-HDA may indicate systemic metabolic remodelling accompanying periodontal healing. This finding suggests that OLE supplementation may exert indirect effects on lipid mediator profiles, warranting further targeted lipidomic investigation.

Taken together, these findings suggest that the metabolic changes observed in the treatment group reflect coordinated modulation of interconnected biological pathways rather than isolated biochemical alterations. The combined increase in metabolites associated with antioxidant activity (e.g., cinnamic acid), tissue metabolism and repair processes (e.g., valine), and anti-inflammatory lipid mediators (e.g., 10-HDA), together with reduced stress-related responses, supports a systemic shift toward a pro-resolving and regenerative physiological environment. Such coordinated metabolic adaptations may contribute to immune regulation, attenuation of oxidative stress burden, and improved tissue homeostasis, all of which are central to periodontal healing. In addition, the potential involvement of microbiota-derived or microbiota-modulated metabolites suggests that olive leaf extract supplementation may influence host–microbiota metabolic interactions, further supporting a systems-level mechanism underlying the observed clinical improvements.

It is also important to consider that metabolic changes may precede measurable clinical improvements. Systemic metabolic responses can represent early biological adaptations to intervention that occur before macroscopic clinical outcomes become fully evident. Therefore, the observed metabolomic shifts may reflect early indicators of improved inflammatory regulation and tissue repair capacity, potentially predicting longer-term clinical benefits beyond the duration of the present study. This perspective may help explain the modest magnitude of some clinical changes despite clear metabolic modulation.

The metabolomic profile observed in the treatment group further supports a systemic role of OLE in modulating host responses relevant to periodontal outcomes. Beyond providing mechanistic insights, metabolomic profiling offers a framework to identify biochemical signatures associated with therapeutic response and to better understand the systemic effects of adjunctive nutraceutical interventions. In addition to the present findings, the OLIVAGING trial also collected gingival crevicular fluid and faecal samples for future analysis of local and systemic responses (Forbes-Hernández et al., 2025), together with broader biochemical and microbiological assessments. These complementary datasets, along with structural characterization of unidentified metabolites, will further clarify the biological actions of olive leaf extract. An integrative conceptual pathway model summarizing the proposed links between oleuropein supplementation, systemic metabolic adaptation, and periodontal outcomes is presented in Fig. 10.

This study has several limitations that should be acknowledged. The relatively small sample size and the modest magnitude of some clinical improvements require cautious interpretation of the findings. Although attrition reached approximately 28 %, no withdrawals were associated with adverse events, and discontinuations were slightly more frequent in the placebo group, suggesting that tolerability of the oleuropein-enriched extract was not a limiting factor. In addition, the untargeted metabolomic approach is inherently exploratory. Several metabolites were tentatively identified based on accurate mass and database matching, without confirmation by authentic standards, which introduces a degree of identification uncertainty. Furthermore, untargeted LC–MS metabolomics provides incomplete coverage of the metabolome and is influenced by extraction procedures, chromatographic conditions, and ionization efficiency, potentially biasing detection toward specific metabolite classes. The analysis was based on relative abundance changes rather than absolute quantification and therefore does not allow direct inference of systemic concentrations. The study duration may also limit the assessment of longer-term clinical effects. Future larger and longitudinal studies, including targeted quantitative metabolomics and mechanistic validation, are warranted to confirm these findings and further clarify the mechanistic pathways involved.

Conclusions

Adjunctive supplementation with a standardized oleuropein-enriched olive leaf extract improved periodontal outcomes in older adults, producing greater reductions in probing depth and modest gains in clinical attachment compared with standard therapy alone. These clinical effects were accompanied by distinct systemic metabolomic shifts, with several metabolites associated with inflammatory regulation, oxidative stress balance, and tissue homeostasis correlating with periodontal improvements. The coordinated metabolic changes observed are consistent with a systemic modulation of interconnected biological pathways involved in immune regulation, oxidative stress attenuation, and host metabolic responses. While these mechanistic interpretations are hypothesis-generating and require targeted validation, the findings support the value of integrating metabolomic profiling with clinical assessment to better understand adjunctive nutraceutical interventions and to identify potential early biomarkers of therapeutic response. Overall, this study supports the potential of oleuropein-enriched olive leaf extract as a safe and multi-target adjunctive strategy for periodontitis management in older adults. However, the modest magnitude of clinical improvements and the relatively small sample size warrant cautious interpretation, and larger, longer-term studies are required to confirm clinical efficacy and further elucidate the mechanistic pathways involved.

Author agreement

I, the undersigned declare that this manuscript en6tled “Adjuvant Treatment with Olive Leaf Extract Enriched in Oleuropein Improves Oral Health Outcomes in Older Adults with Periodon66s: Metabolomic Insights from a Randomized Controlled Trial” is original, has not been published before and is not currently being considered for publica6on elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are not other persons who sa6sfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the corresponding author is the sole contact for the Editorial process. He is responsible for communica6ng with the other authors about progress, submissions of revisions and final approval of proofs.

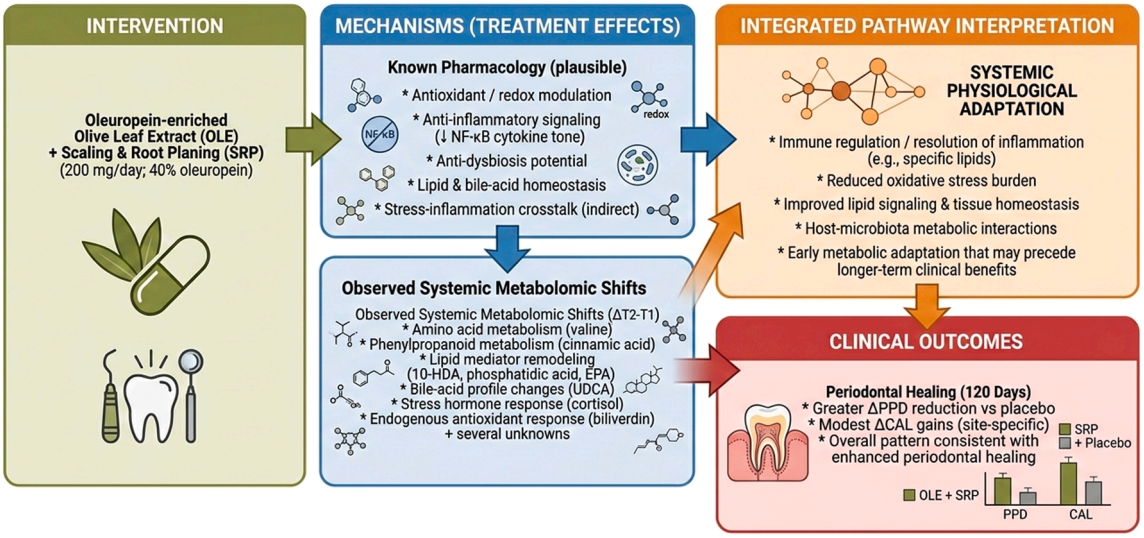


Fig. 10. Conceptual integrative model linking oleuropein supplementation, systemic metabolomic shifts, and periodontal outcomes. Schematic representation of the proposed links between oleuropein-enriched olive leaf extract administered as an adjunct to non-surgical periodontal therapy, its known pharmacological properties (antioxidant, anti-inflammatory, lipid and bile-acid modulation, and potential host–microbiota interactions), the observed systemic metabolomic changes in the Δ (T2–T1) analysis, and the resulting improvements in ΔPPD and ΔCAL after 120 days. Arrows indicate hypothesized biological connections integrating established pharmacology with the metabolomic associations detected in this study and do not imply direct causality.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT-5 (OpenAI, San Francisco) to help with language editing and manuscript organization. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRediT authorship contribution statement

Tamara Y. Forbes-Hernández: Conceptualization. **Franklin G. Vargas-Corral:** Methodology, Investigation. **Beatriz Bullón:** Methodology, Investigation, Formal analysis. **Lorenzo Rivas-García:** Methodology, Investigation, Formal analysis. **Vivian Lipari:** Validation, Data curation. **Francesca Giampieri:** Validation, Formal analysis, Data curation. **Giuseppe Grosso:** Validation, Data curation. **Maurizio Battino:** Supervision, Conceptualization. **Pedro Bullón:** Supervision, Investigation, Funding acquisition, Conceptualization. **José L. Quiles:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by the grant PID2019-106778RB-I00, funded by MCIN/AEI/10.13039/501100011033 FEDER “Una manera de hacer Europa”, and by the “Visiting Scholars 2022” Program from the Universidad de Granada. Funding for open access charge: Universidad de Granada / CBUA.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2026.158222.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Alves, F., Stringasci, M.D., Requena, M.B., Blanco, K.C., Dias, L.D., Corrêa, T.Q., Bagnato, V.S., 2022. Randomized and controlled clinical studies on antibacterial photodynamic therapy: an overview. Photonics 9. https://doi.org/10.3390/photonics9050340.

Baima, G., Romandini, M., Citterio, F., Romano, F., Aimetti, M., 2022. Periodontitis and accelerated biological aging: a geroscience approach. J. Dent. Res. 101, 125–132. https://doi.org/10.1177/00220345211037977.

Benameur, T., Porro, C., Twfieg, M.-E., Benameur, N., Panaro, M.A., Filannino, F.M., Hasan, A., 2023. Emerging paradigms in inflammatory disease management: exploring bioactive compounds and the gut microbiota. Brain Sci. 13, 1226. https://doi.org/10.3390/brainsci13081226.

Biernacki, M., Skrzydlewska, E., 2025. Metabolic pathways of eicosanoids-derivatives of arachidonic acid and their significance in skin. Cell Mol. Biol. Lett. 30, 7. https://doi.org/10.1186/s11658-025-00685-y.

Boehm, T.K., Scannapieco, F.A., 2007. The epidemiology, consequences and management of periodontal disease in older adults. J. Am. Dent. Assoc. 138, S26–S33. https://doi.org/10.14219/jada.archive.2007.0360.

Bullon, P., Giampieri, F., Bullon, B., Battino, M., 2025. The role of oxidative stress in periodontitis. J. Periodontal Res. https://doi.org/10.1111/jre.70016.

Bullon, P., Morillo, J.M., Ramirez-Tortosa, M.C., Quiles, J.L., Newman, H.N., Battino, M., 2009. Metabolic syndrome and periodontitis: is oxidative stress a common link? J. Dent. Res. 88, 503–518. https://doi.org/10.1177/0022034509337479.

Chen, Y.-F., Wang, K., Zhang, Y.-Z., Zheng, Y.-F., Hu, F.-L., 2016. Vitro anti-inflammatory effects of three fatty acids from royal jelly. Mediators Inflamm., 3583684 https://doi.org/10.1155/2016/3583684, 2016.

Forbes-Hernández, T.Y., Vargas-Corral, F., Rivas-García, L., Bullón, B., Varela-López, A., Armas-Diaz, Y., Romero-Márquez, J.M., Navarro-Hortal, M.D., Bullón, P., Quiles, J. L., 2025. Addressing the physiopathology of periodontitis in older adults using an oleuropein-rich olive leaf supplement: study design and baseline characteristics of the OLIVAGING clinical trial. Mediterr. J. Nutr. Metab. 18, 215–223. https://doi.org/10.1177/1973798×251357485.

Genco, R.J., Sanz, M., 2020. Clinical and public health implications of periodontal and systemic diseases: an overview. Periodontol. 2000 83, 7–13. https://doi.org/10.1111/prd.12344.

Gonzalez-Covarrubias, V., Martínez-Martínez, E., Del Bosque-Plata, L., 2022. The potential of metabolomics in biomedical applications. Metabolites 12, 194. https://doi.org/10.3390/metabo12020194.

Gonzalez-Sanchez, E., Perez, M.J., Nytofte, N.S., Briz, O., Monte, M.J., Lozano, E., Serrano, M.A., Marin, J.J.G., 2016. Protective role of biliverdin against bile acid-

induced oxidative stress in liver cells. Free Radic. Biol. Med. 97, 466–477. https://doi.org/10.1016/j.freeradbiomed.2016.06.016.
Hajishengallis, G., Chavakis, T., 2021. Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. Nat. Rev. Immunol. 21, 426–440. https://doi.org/10.1038/s41577-020-00488-6.
Hammami, C., Nasri, W., 2021. Antibiotics in the treatment of periodontitis: a systematic review of the literature. Int. J. Dent., 6846074 https://doi.org/10.1155/2021/6846074, 2021.
Herrera, D., Matesanz, P., Martín, C., Oud, V., Feres, M., Teughels, W., 2020. Adjunctive effect of locally delivered antimicrobials in periodontitis therapy: a systematic review and meta-analysis. J. Clin. Periodontol. 47 (Suppl 22), 239–256. https://doi.org/10.1111/jcpe.13230.
Hu, X., Liu, Z., Lu, Y., Chi, X., Han, K., Wang, H., Wang, Y., Ma, L., Xu, B., 2022. Glucose metabolism enhancement by 10-hydroxy-2-decenoic acid via the PI3K/AKT signaling pathway in high-fat-diet/streptozotocin induced type 2 diabetic mice. Food Funct. 13, 9931–9946. https://doi.org/10.1039/D1FO03818D.
Karygianni, L., Cecere, M., Skaltsounis, A.L., Argyropoulou, A., Hellwig, E., Aligiannis, N., Wittmer, A., Al-Ahmad, A., 2014. High-level antimicrobial efficacy of representative Mediterranean natural plant extracts against oral microorganisms. BioMed Res. Int., 839019 https://doi.org/10.1155/2014/839019, 2014.
Kondreddy, K., Mohanty, A., Desai, V., Surekha, G.L., Khader, A.A., Mathur, S., Shetty, S., 2025. The effect of adjunctive antimicrobial therapy on the clinical outcomes of non-surgical periodontal treatment: a systematic review. J. Pharm. Bioallied Sci. 17, S463. https://doi.org/10.4103/jpbs.jpbs_1426_24.
Lillis, J.B., Willmott, A.G.B., Chichger, H., Roberts, J.D., 2025. The application of olive-derived polyphenols on exercise-induced inflammation: a scoping review. Nutrients 17, 223. https://doi.org/10.3390/nu17020223.
Morey, J.N., Boggero, I.A., Scott, A.B., Segerstrom, S.C., 2015. Current directions in stress and Human immune function. Curr. Opin. Psychol. 5, 13–17. https://doi.org/10.1016/j.copsyc.2015.03.007.
Moura, M.F., Cota, L.O.M., Silva, T.A., Cortelli, S.C., Ferreira, G.A., López, M.M., Cortelli, J.R., Costa, F.O., 2021. Clinical and microbiological effects of non-surgical periodontal treatment in individuals with rheumatoid arthritis: a controlled clinical trial. Odontology 109, 484–493. https://doi.org/10.1007/s10266-020-00566-0.
Ogwu, M.C., Izah, S.C., Joshua, M.T., 2025. Ecological and environmental determinants of phytochemical variability in forest trees. Phytochem. Rev. https://doi.org/10.1007/s11101-025-10066-0.
Omar, S.H., 2010. Oleuropein in olive and its pharmacological effects. Sci. Pharm. 78, 133–154. https://doi.org/10.3797/scipharm.0912-18.
Pang, Z., Lu, Y., Zhou, G., Hui, F., Xu, L., Viau, C., Spigelman, A.F., MacDonald, P.E., Wishart, D.S., Li, S., Xia, J., 2024. MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation. Nucleic Acids Res. 52, W398–W406. https://doi.org/10.1093/nar/gkae253.
Polizzi, A., Alibrandi, A., Lo Giudice, A., Distefano, A., Orlando, L., Analazi, A.M., Pizzo, G., Volti, G.L., Isola, G., 2024. Impact of periodontal microRNAs associated with alveolar bone remodeling during orthodontic tooth movement: a randomized clinical trial. J. Transl. Med. 22, 1155. https://doi.org/10.1186/s12967-024-05933-x.
Polizzi, A., Nibali, L., Tartaglia, G.M., Isola, G., 2025. Impact of nonsurgical periodontal treatment on arterial stiffness outcomes related to endothelial dysfunction: a systematic review and meta-analysis. J. Periodontol. 96, 330–345. https://doi.org/10.1002/JPER.24-0422.
Preshaw, P.M., Hefti, A.F., Jepsen, S., Etienne, D., Walker, C., Bradshaw, M.H., 2004. Subantimicrobial dose doxycycline as adjunctive treatment for periodontitis. A review. J. Clin. Periodontol. 31, 697–707. https://doi.org/10.1111/j.1600-051X.2004.00558.x.
Romero-Márquez, J.M., Navarro-Hortal, M.D., Jiménez-Trigo, V., Vera-Ramírez, L., Forbes-Hernández, T.J., Esteban-Muñoz, A., Giampieri, F., Bullón, P., Battino, M., Sánchez-González, C., Quiles, J.L., 2022. An oleuropein rich-olive (Olea europaea L.) leaf extract reduces β-amyloid and tau proteotoxicity through regulation of oxidative- and heat shock-stress responses in Caenorhabditis elegans. Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 162, 112914. https://doi.org/10.1016/j.fct.2022.112914.
Ruwizhi, N., Aderibigbe, B.A., 2020. Cinnamic acid derivatives and their biological efficacy. Int. J. Mol. Sci. 21, 5712. https://doi.org/10.3390/ijms21165712.
Sanz, I., Alonso, B., Carasol, M., Herrera, D., Sanz, M., 2012. Nonsurgical treatment of periodontitis. J. Evid.-Based Dent. Pract. 12, 76–86. https://doi.org/10.1016/S1532-3382(12)70019-2.
Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: communicating confidence. Environ. Sci. Technol. 48, 2097–2098. https://doi.org/10.1021/es5002105.
Shah, S.H., Newgard, C.B., 2015. Integrated metabolomics and genomics: systems approaches to biomarkers and mechanisms of cardiovascular disease. Circ. Cardiovasc. Genet. 8, 410–419. https://doi.org/10.1161/CIRCGENETICS.114.000223.
Spahr, A., Divnic-Resnik, T., 2022. Impact of health and lifestyle food supplements on periodontal tissues and health. Periodontol. 2000 90, 146–175. https://doi.org/10.1111/prd.12455.
Teughels, W., Feres, M., Oud, V., Martín, C., Matesanz, P., Herrera, D., 2020. Adjunctive effect of systemic antimicrobials in periodontitis therapy: a systematic review and meta-analysis. J. Clin. Periodontol. 47 (Suppl 22), 257–281. https://doi.org/10.1111/jcpe.13264.
Varela-López, A., Bullón, P., Giampieri, F., Quiles, J.L., 2015. Non-nutrient, naturally occurring phenolic compounds with antioxidant activity for the prevention and treatment of periodontal diseases. Antioxidants 4, 447–481. https://doi.org/10.3390/antiox4030447.
Vieira, S.F., Reis, R.L., Ferreira, H., Neves, N.M., 2025. Plant-derived bioactive compounds as key players in the modulation of immune-related conditions. Phytochem. Rev. 24, 343–460. https://doi.org/10.1007/s11101-024-09955-7.
Yahsi, B., Gunaydin, G., 2022. Immunometabolism – The role of branched-chain amino acids. Front. Immunol. 13, 886822. https://doi.org/10.3389/fimmu.2022.886822.