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Protective Effects Induced by a Novel Food Supplement Based on *Epilobium angustifolium*, *Ptychopetalum olacoides*, *Ginkgo biloba*, and *Equisetum arvense* Extracts and Citrulline in the Lower Urinary Tract: Preclinical and Clinical Evidence

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ABSTRACT

Oxyfil is a dietary supplement including plant extracts from *Epilobium angustifolium*, *Ptychopetalum olacoides* (*Muiria puama*), *Ginkgo biloba*, and *Equisetum arvense* and citrulline. The aim of the study was to investigate the protective effects on prostatic muscles of the Oxyfil formulation in an in vitro experimental model consisting of isolated mouse prostate specimens exposed to *Escherichia coli* lipopolysaccharide (LPS) as a pro-inflammatory stimulus. The study also assessed the tolerability and effectiveness of Oxyfil in alleviating lower urinary tract symptoms (LUTS) in patients with benign prostatic hyperplasia (BPH) when used in association with standard care. In the preclinical phase, Oxyfil was analysed via liquid chromatographic in order to identify and quantify phenolic compounds. Thereafter, the formulation was tested on isolated mouse prostate challenged with LPS in order to upregulate the gene expression of different pro-inflammatory and oxidative stress biomarkers, namely COX-2, i-NOS, TNF α , IL-6, VEGFA, HIF1 α . The clinical trial was a retrospective, multicentre study. Fifty-nine patients were analyzed: 27 received Oxyfil, 18 Oxyfil + α -blocker, 7 Oxyfil + PDE5i, and 7 Oxyfil + 5-ARI. Key inclusion criteria were age 50–75 years, moderate to severe LUTS (IPSS), mild erectile dysfunction, and baseline $Q_{\max}$ 4–15 mL/s; exclusions were endocrine/hormonal erectile dysfunction, bladder-neck sclerosis, and urethral stricture. Treatment selection was non-randomized and left to the treating physician. The chromatographic analysis identified 25 phenolic compounds in the Oxyfil formulation. The total phenolic content can explain, albeit partially, the Oxyfil efficacy in blunting LPS-induced upregulation of the gene expression of all tested biomarkers in isolated prostate. The protective effects demonstrated in the preclinical phase also support, albeit partially, the clinical efficacy in ameliorating uroflowmetry and symptom outcomes in the patients' cohort. In conclusion, the present

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study demonstrated anti-inflammatory and antioxidant effects of the Oxyfil formulation in the prostate which corroborated the observed clinical outcomes.

1 | Introduction

In recent years, the relationship between inflammation and the biological characteristics of benign prostatic hyperplasia has attracted considerable academic interest. Evidence from pivotal trials revealed that the risk of lower urinary tract symptoms (LUTS) due to benign prostatic hyperplasia (BPH) is related to the intra-prostatic infiltration of inflammatory cells (Nickel et al. 2008; Torkko et al. 2015).

More studies have shown that inflammation plays a fundamental role not only in chronic abacterial prostatitis but also in the evolution of the symptoms of benign prostatic hyperplasia. The REDUCE study showed that chronic prostatic inflammation can be detected in 77.6% of patients with BPH undergoing prostate biopsy and several studies have shown a significant correlation between chronic prostatic inflammation and the severity of LUTS, prostate volume, and increased risk of acute urinary retention (Kim et al. 2013).

α -Blockers are the primary treatment for LUTS in patients with prostatitis and BPH (Nickel 2006). However, herbal extracts have also been effective in managing inflammation associated with these conditions, and combining them can enhance efficacy (Iglesias-Gato et al. 2012). This aligns with research suggesting that combining plant extracts can benefit chronic inflammatory diseases. Growing interest in alternative treatments, such as nutraceuticals and herbal preparations with potentially fewer side effects, has led to exploring plant extracts used in traditional medicine for managing inflammatory conditions (Jones et al. 2006). These extracts, especially those prepared as traditional infusions or decoctions, could be effective and safe due to their long history of use. They also represent innovative strategies for boosting local botanical productions (Ferrante et al. 2019).

Given the growing use of food supplements, especially for BPH symptoms (Stewart and Lephart 2023), this study aimed to explore the protective effects of Oxyfil, a new dietary supplement, on prostatic muscles. This product combines water extracts from *Epilobium angustifolium*, *Ptychopetalum olacoides*, *Ginkgo biloba*, *Equisetum arvense*, and citrulline. The formulation ingredients have been studied individually and in combination for their potential benefits in addressing LUTS and BPH (Esposito et al. 2021; Moyad 2022; Peng et al. 2013; Schoendorfer et al. 2018), as well as their adaptogenic properties (Piato et al. 2010). Oxyfil was tested in an in vitro experimental model consisting of isolated mouse prostate samples exposed to *Escherichia coli* lipopolysaccharide (LPS) as a pro-inflammatory stimulus. In this context, the gene expression of biomarkers involved in inflammation and oxidative stress, namely cyclooxygenase-2 (COX-2), inducible nitric oxidase (iNOS), tumor necrosis factor α (TNF α), interleukin-6 (IL-6), hypoxia inducible factor 1 α (HIF1 α), and vascular endothelial growth factor A (VEGFA), was also evaluated. Moreover, the study assessed the tolerability and effectiveness of Oxyfil in alleviating LUTS in patients with BPH when used in association with standard care.

2 | Material and Methods

2.1 | Plant Material

The formulation Oxyfil was provided as dried materials by OMEGA PHARMA Srl (Cantù, Italy). The composition includes dry extracts from *Epilobium angustifolium*, *Ptychopetalum olacoides* (*Muiria puama*), *Ginkgo biloba*, *Equisetum arvense*, and L-citrulline. The abovementioned ingredients are present in the formulation at the following percentages, respectively: 27.67, 12.58, 5.66, 22.64, 31.45. The ingredients were reconstituted in water (40 mg/mL) using ultrasound-assisted extraction (Menghini et al. 2018), then diluted in mobile phase and tissue medium for HPLC quantitative analysis and preclinical evaluation in isolated prostate, respectively.

2.2 | HPLC-UV Analysis

The formulation was subjected to reversed-phase HPLC-UV analysis, in gradient elution mode, to quantitatively determine the phenolic composition. The HPLC apparatus consisted of a two PU-2080 PLUS chromatographic pump, a DG-2080-54-line degasser, a mix-2080-32 mixer, UV, an AS-2057 PLUS autosampler, and a CO-2060 PLUS column thermostat (all from Jasco, Tokyo, Japan). ChromNAV2 Chromatography software was used for integration. The separation was conducted within 48 min of the chromatographic run, starting from the following conditions: 97% water with 0.1% formic acid, and 3% methanol with 0.1% formic acid. The flow was set at 0.6 mL/min throughout the analysis Table 1. The analytes were separated on an Infinity lab

TABLE 1 | Gradient elution condition for chromatographic determination (HPLC-UV) analysis of phenolic compounds. UV detector was set at 254 nm.

Time (min)	Composition A% (Water + Formic acid 0.1%)	Composition B% (Methanol + Formic acid 0.1%)	Flow (mL/min)
1	97	3	0.6
5	77	23	0.6
12	73	27	0.6
18	57	43	0.6
25	52	48	0.6
32	50	50	0.6
34	50	50	0.6
37	35	65	0.6
40	5	95	0.6
47	10	90	0.6
48	10	90	0.6

Poroshell 120-SB reverse phase column (C18, 150×4.6 mm i.d., 2.7µm; Agilent, Santa Clara, CA, USA). Column temperature was set at 30°C. Quantitative determination of phenolic compounds was performed via UV detector at 254 nm. The injection volume was 5 µL. Quantification was performed through 7-point calibration curves, with linearity coefficients (R^2) > 0.999, in the concentration range 2–140 µg/mL. The final concentration of the extract was 40 mg/mL before injecting in HPLC system.

2.3 | Colorimetric Assays

The total phenolic and flavonoid contents were quantified using the Folin–Ciocalteu colorimetric method. Results were expressed as gallic acid equivalents (mg GAE/g dry extract) for phenolics and rutin equivalents (mg RE/g dry extract) for flavonoids. The antioxidant potential of the extracts was evaluated through ABTS assay. Detailed experimental protocols have been previously described in the literature (Atasagun et al. 2025).

2.4 | In Vitro Studies

Adult C57/BL6 and male mice (3-month-old, weight 20–25 g) were housed in Plexiglass cages (2–4 animals per cage; 55×33×19 cm) and maintained under standard laboratory conditions (21°C ± 2°C; 55% ± 5% humidity) on a 14/10 h light/dark cycle, with ad libitum access to water and food. Housing conditions and experimentation procedures were strictly in agreement with the European Community ethical regulations (EU Directive no. 26/2014) on the care of animals for scientific research. In agreement with the recognized principles of “Replacement, Refinement and Reduction in Animals in Research”, prostate specimens were obtained as residual material from vehicle-treated mice randomized in our previous experiments, approved by local ethical committee (‘G. d’Annunzio’ University, Chieti, Italy) and Italian Health Ministry (Project no. 885/2018-PR).

Isolated prostate specimens were maintained in a humidified incubator with 5% CO₂ at 37°C for 4 h (incubation period), in RPMI buffer with added bacterial LPS (50 µg/mL). During the incubation period, the tissues were challenged with scalar concentrations of the extract (200–1000 µg/mL).

2.5 | Gene Expression Analysis

Total RNA was extracted from both prostate and ovary specimens using TRI reagent (Sigma-Aldrich, St. Louis, MO, USA), according to the manufacturer’s protocol, and reverse transcribed using High Capacity cDNA Reverse Transcription Kit (ThermoFischer Scientific, Waltham, Massachusetts, USA). Gene expression of TNFα, IL-6, VEGFA, HIF1α, COX-2 and SOD was determined by quantitative real-time PCR using TaqMan probe-based chemistry. PCR primers and TaqMan probes were purchased from Thermo Fisher Scientific Inc. The elaboration of data was conducted with the Sequence Detection System (SDS) software version 2.3 (ThermoFischer Scientific). Relative quantification of gene expression was performed by the comparative 2^{−ΔΔCt} method.

2.6 | In Vivo Study—Patients’ Selection

This is a retrospective, multicentre, non-interventive study. Consecutive men with LUTS due to BPH were managed under routine care. At the baseline (pre-treatment) assessment, Oxyfil was initiated for all patients—either as an add-on to ongoing standard therapy (alpha-blocker, 5-ARI, or PDE5 inhibitor) or, in previously untreated patients, as de novo monotherapy or combined therapy. The Oxyfil was prescribed as 1 pill per day for at least 8 weeks, and a routine follow-up was scheduled. Treatment allocation was consecutive, non-randomized, and clinician driven.

Key inclusion criteria were age 50–75 years, moderate to severe LUTS (IPSS), mild erectile dysfunction, and baseline Q_{max} 4–15 mL/s; exclusions were endocrine/hormonal erectile dysfunction, bladder-neck sclerosis, and urethral stricture.

Data were collected at baseline and at routine post-treatment follow-up. Recorded variables included age, BMI, comorbidities, uroflowmetry (Q_{max}), post-void residual (PVR), IPSS total, and IIEF-5. The primary endpoint was change in IPSS (post-pre). Secondary endpoints were changes in Q_{max} , PVR, and IIEF-5. An exploratory domain analysis decomposed IPSS into voiding subscores (1, 3, 5, 6) and storage subscores (2, 4, 7); for each patient and treatment group we computed pre/post domain sums and their deltas (post-pre).

2.7 | Statistical Analysis

Statistical analysis was performed by GraphPad Prism (Version 5.01) software (GraphPad Software Inc., San Diego, CA, USA). In the preclinical phase, data were evaluated through analysis of variance (ANOVA) followed by Newman–Keuls comparison multiple test. The statistical significance was set at $p < 0.05$.

Regarding the clinical study, baseline group comparisons used Kruskal–Wallis (continuous) and Pearson’s chi-square (categorical), reported as median (IQR) or counts (%). Pre/post changes were tested with paired Wilcoxon tests. For domain analysis, paired Wilcoxon tests were run within each domain, and a paired comparison of deltas (storage minus voiding subscores) was performed per treatment group; when all paired differences were zero the test was deemed non-informative. Two-sided $\alpha = 0.05$; no multiplicity adjustment given the exploratory aims. Analyses were performed in R (v4.4.2) using tidyverse, janitor, arsenal (tableby), ggplot2/ggpubr/ggsci, and base functions (wilcox.test, lm).

3 | Results

3.1 | Chemical Composition

The formulation was subjected to a qualitative and quantitative HPLC–UV analysis to determine its phenolic composition. A total of 25 compounds were identified (Figure 1). Quantitative analysis showed that benzoic acid, chlorogenic acid, rutin, t-ferulic acid, naringenin, and resveratrol were among the most

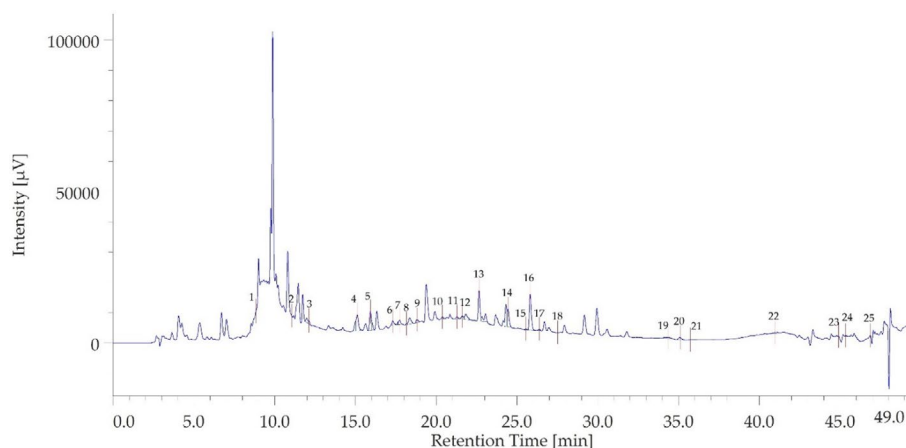


FIGURE 1 | Chromatogram of the Oxyfil formulation. Among the identified phenolics, the prominent analytes were gallic acid (Rt 8.2 min, peak n.1), hydroxytyrosol (Rt 11.2 min, peak n.2), caffeic acid (Rt 12.2 min, peak n.3), 4-hydroxybenzoic acid (Rt 15.1 min, peak n.4), chlorogenic acid (Rt 15.9 min, peak n.5), vanillic acid (Rt 17.3 min, peak n.6), caffeic acid (Rt 17.7 min, peak n.7), epicatechin (Rt 18.2 min, peak n.8), synaptic acid (Rt 18.8 min, peak n.9), syringaldehyde (Rt 20.4 min, peak n.10), chicoric acid (Rt 21.3 min, peak n.11), p-coumaric acid (Rt 21.6 min, peak n.12), t-ferulic acid (Rt 22.6 min, peak n.13), benzoic acid (Rt 24.4 min, peak n.14), hyperoside (Rt 25.5 min, peak n.15), rutin (Rt 25.8 min, peak n.16), resveratrol (Rt 26.4 min, peak n.17), rosmarinic acid (Rt 27.5 min, peak n.18), quercetin (Rt 34.3 min, peak n.19), naringenin (Rt 35.1 min, peak n.20), kaempferol (Rt 41.0 min, peak n.22), and other minor phenolics.

abundant compounds in the formulation (Table 2). The formulation's botanical ingredients, namely *Epilobium angustifolium*, *Ptychopetalum olacoides*, *Ginkgo biloba*, and *Equisetum arvense* extracts, were also assayed for total phenolic (TPC), total flavonoid (TFC), and antiradical effect (ABTS assay). The results showed that *G. biloba* extract was the richest source of TPC and TFC of the formulation (Table 3). This was also in agreement with its highest potency as an antiradical agent in the ABTS assay (Table 4).

3.2 | Protective Effects in Isolated Prostate

Exposure of mouse prostate specimens to LPS (50 µg/mL) resulted in a significant induction of all assayed pro-inflammatory biomarkers, including COX-2, iNOS, IL-6, TNFα, VEGFA, and HIF1α. Treatment with the Oxyfil formulation at concentrations ranging from 200 to 1000 µg/mL blunted this response across all markers.

Specifically, Oxyfil significantly reduced COX-2 gene expression compared with the LPS group (ANOVA, $p < 0.0001$; Figure 2A), with comparable inhibition observed at 200, 500, and 1000 µg/mL. A similar pattern was detected for IL-6 (ANOVA, $p < 0.0001$; Figure 2B) and TNFα (ANOVA, $p < 0.0001$; Figure 2C), where all concentrations markedly suppressed the LPS-induced transcriptional increase.

Oxyfil also significantly reduced the expression of angiogenic markers VEGFA (ANOVA, $p < 0.0001$; Figure 2D) and HIF1α (ANOVA, $p < 0.0001$; Figure 2E) in LPS-challenged prostate tissue, with the highest concentration (1000 µg/mL) showing the greatest attenuation. Finally, a consistent reduction in iNOS expression was observed at all concentrations (ANOVA, $p < 0.0001$; Figure 2F). Across all endpoints, Oxyfil restored biomarker levels toward those of untreated controls, with no evidence of concentration-related toxicity in the tissue model.

3.3 | In Vivo Study

Fifty-nine patients were analyzed: 27 received Oxyfil, 18 Oxyfil + α-blocker, 7 Oxyfil + PDE5i, and 7 Oxyfil + 5-ARI. Baseline prostate volume differed across groups and was larger in the Oxyfil + 5-ARI cohort (median ~70 mL) compared with the others (overall $p < 0.001$; Table 5).

After a median of 90 days of exposure to Oxyfil, in the overall cohort, uroflowmetry and symptom outcomes improved significantly after treatment (Table 6). Findings were consistent in the Oxyfil-only subgroup: $Q_{\max}$ increased by median of +3.0 mL/s ($p < 0.001$), PVR reduced by median −40 mL ($p < 0.001$), IPSS decreased as median of −4 points ($p < 0.001$) and IIEF also increased (+4.0; $p < 0.001$).

In an exploratory domain analysis of IPSS (Δ defined as post-pre), voiding symptoms decreased more than storage symptoms in three groups: Oxyfil (paired Wilcoxon across domains $p < 0.001$), Oxyfil + alpha-blocker ($p < 0.001$), and Oxyfil + 5-ARI ($p < 0.001$). In Oxyfil + PDE5i the paired comparison was not informative due to near-constant Δ values.

4 | Discussion

The current study showed an active anti-inflammatory activity of the Oxyfil formulation in prostate tissues, reflecting in an improvement of main uroflowmetry and prostate symptoms.

The phenolic composition of the formulation provides a plausible mechanistic rationale for its biological activity. Hydroxycinnamic acids, benzoic acids, flavonoids, and stilbenes—quantified through HPLC-UV—have been individually associated with antioxidant and anti-inflammatory properties in preclinical models (Ferrante et al. 2020; Graefe and Veit 1999; Nowak et al. 2021; Rolim et al. 2006; Rusak et al. 2024).

In the in vitro prostate inflammation system, exposure to LPS induced a marked transcriptional increase in COX-2, iNOS, IL-6, TNF- α , VEGFA, and HIF1 α , reflecting the expected inflammatory and oxidative environment (Ferrante et al. 2020; Inamura and Terada 2024). Across all concentrations tested, the formulation

TABLE 2 | Retention times (min) and quantities ($\mu\text{g/mL}$) of the investigated phenolic compounds (nq = not quantified).

	Analytes	Rt (min)	Quantity ($\mu\text{g/mL}$)
1	Gallic acid	8.9	0.128 ± 0.020
2	Hydroxytyrosol	11.1	0.156 ± 0.001
3	Caftaric acid	12.2	1.197 ± 0.266
4	4-Hydroxybenzoic acid	15.1	0.802 ± 0.072
5	Chlorogenic acid	15.9	6.898 ± 0.140
6	Vanillic acid	17.3	0.379 ± 0.171
7	Caffeic acid	17.7	0.663 ± 0.149
8	Epicatechin	18.2	1.859 ± 0.519
9	Syringic acid	18.8	1.109 ± 0.185
10	Syringaldehyde	20.4	2.638 ± 0.114
11	Chicoric acid	21.3	0.479 ± 0.060
12	p-Coumaric acid	21.6	1.003 ± 0.344
13	t-Ferulic acid	22.6	5.310 ± 0.103
14	Benzoic acid	24.4	14.432 ± 0.624
15	Hyperoside	25.5	0.330 ± 0.003
16	Rutin	25.8	6.730 ± 0.108
17	Resveratrol	26.4	2.724 ± 0.005
18	Rosmarinic acid	27.5	0.317 ± 0.005
19	Quercetin	34.3	1.751 ± 0.184
20	Naringenin	35.1	2.085 ± 1.237
21	2,3-Dimethylbenzoic acid	35.7	0.801 ± 0.207
22	Kaempferol	41.0	1.753 ± 0.044
23	Flavone	44.9	nq
24	3-Hydroxyflavone	45.3	0.944 ± 0.043
25	Emodin	46.9	0.743 ± 0.133

attenuated the LPS-induced upregulation of these biomarkers. The direction and consistency of these reductions align with the phenolic pattern identified analytically, supporting the biological plausibility of the formulation's anti-inflammatory profile (Chen et al. 2019; Orlando, Chiavaroli, Adorisio, et al. 2021; Orlando, Chiavaroli, Ferrante, et al. 2021). Among the assayed polyphenols, benzoic acid, hydrocinnamic acids, such as chlorogenic and ferulic acid, and flavonoids, among which rutin, are the main phytochemicals quantified in the formulation. Scientific literature pointed to inhibitory effects displayed by these compounds on the activity of the assayed pro-inflammatory biomarkers. Indeed, chlorogenic acid was found effective, in vitro, in inhibiting the gene expression of COX-2, i-NOS, and cytokines induced by pro-inflammatory stimuli (Chen and Wu 2014; Huang et al. 2023; Shan et al. 2009). Intriguingly, a similar anti-inflammatory profile was reported as regards ferulic acid that was able to revert the increased synthesis of TNF α , IL-6, i-NOS, and COX-2 in the testis of rats challenged with a pro-inflammatory stimulus, namely 2-methoxyethanol (Adeyi et al. 2023). Moreover, benzoic acids and rutin derivatives were found effective in inhibiting COX-2 (Ahmed et al. 2024), whereas rutin was also able to inhibit the protein and gene levels of pro-inflammatory cytokines, among which TNF α and IL-6 (Jang et al. 2025). The pattern in phenolic compounds displayed by the formulation could also explain, albeit in part, the gene expression inhibition of both VEGFA and HIF α (Ansó et al. 2010; Lin et al. 2017), two well-known angiogenesis-stimulating factors (Kang et al. 2023), that are deeply involved in the so-called inflammatory-to-cancer transition (Chen et al. 2019).

Overall, the present results demonstrated the anti-inflammatory and antioxidant effects of the formulation in an in vitro experimental model of bacterial prostatitis or inflammation induced by benign prostatic hyperplasia. The protective effects induced by the formulation are consistent, albeit in part, with its content of phenolic compounds, particularly hydroxycinnamic acids and flavonoids. The efficacy demonstrated by the formulation supports clinical evaluation in patients with benign prostatic hyperplasia, when underlying inflammation induces increased urethral stiffness and the appearance of obstructive urinary symptoms (Zhang et al. 2020).

Taken together, the preclinical and phytochemical data suggest that the formulation exerts measurable anti-inflammatory effects in prostate tissue, providing a mechanistic foundation for clinical evaluation in patients with LUTS.

Such effect was confirmed in vivo. Indeed, treatment with Oxyfil—alone or in combination with standard therapies—was associated with improvements in uroflowmetry, residual

TABLE 3 | Total phenolic content and flavonoid content of the extracts under study.

Sample	TPC (GAE) (mg/g_{de})	TFC (RE) (mg/g_{de})
<i>Epilobium angustifolium</i>	23.69 ± 0.78	0.90 ± 0.22
<i>Equisetum arvense</i>	10.89 ± 0.28	n.d.
<i>Ptychopetalum olacoides</i>	8.80 ± 0.39	n.d.
<i>Ginkgo biloba</i>	40.73 ± 1.35	23.80 ± 1.33

Note: Values are reported as mean $\pm$ S.D. of three parallel measurements.

Abbreviations: de, dry extract; GAE, Gallic acid equivalents; n.d., not determined; RE, Rutin equivalents; TFC, Total flavonoid content; TPC, Total phenolic content.

urine and symptom burden; thus, further supporting the use of botanicals-based food supplements in reducing the burden of inflammation and oxidative stress and improving urinary $Q_{\max}$ in LUTS (Orlando, Chiavaroli, Adorisio, et al. 2021; Orlando, Chiavaroli, Ferrante, et al. 2021). However, we have also to consider that about 30% formulation is constituted by the amino acid citrulline, that is converted in NO oxide, in the kidney (Papadia

et al. 2018) and that could influence, albeit in part, the observed clinical outcomes (Yagi et al. 2017). Indeed, amino acids like citrulline and arginine, involved in nitric oxide biosynthesis, are effective in controlling BPH symptoms (Moyad 2022), also in association with medicinal plants (Yagi et al. 2017).

Exploratory decomposition of IPSS showed that the overall symptom reduction was driven predominantly by voiding rather than storage subscores in three of the four treatment groups. However, baseline heterogeneity warrants cautious interpretation. Prostate volume differed substantially across groups and was highest in patients receiving 5-ARI, introducing the possibility of residual confounding in a retrospective, clinician-driven allocation framework. This factor may influence both urinary flow trajectories and symptom burden.

Despite these limitations, the overall pattern suggests that initiating Oxyfil at the pre-treatment visit—whether as monotherapy or add-on—may augment short-term improvements in voiding function and symptoms, particularly in patients with

TABLE 4 | Antioxidant properties of the extracts under study.

Sample	ABTS IC ₅₀ (mg/mL)
<i>Epilobium angustifolium</i>	2.46 ± 0.01
<i>Equisetum arvense</i>	5.72 ± 0.07
<i>Ptychopetalum olacoides</i>	5.79 ± 0.70
<i>Ginkgo biloba</i>	0.87 ± 0.01
Trolox	0.03 ± 0.01

Note: Values are reported as mean ± S.D. of three parallel measurements.

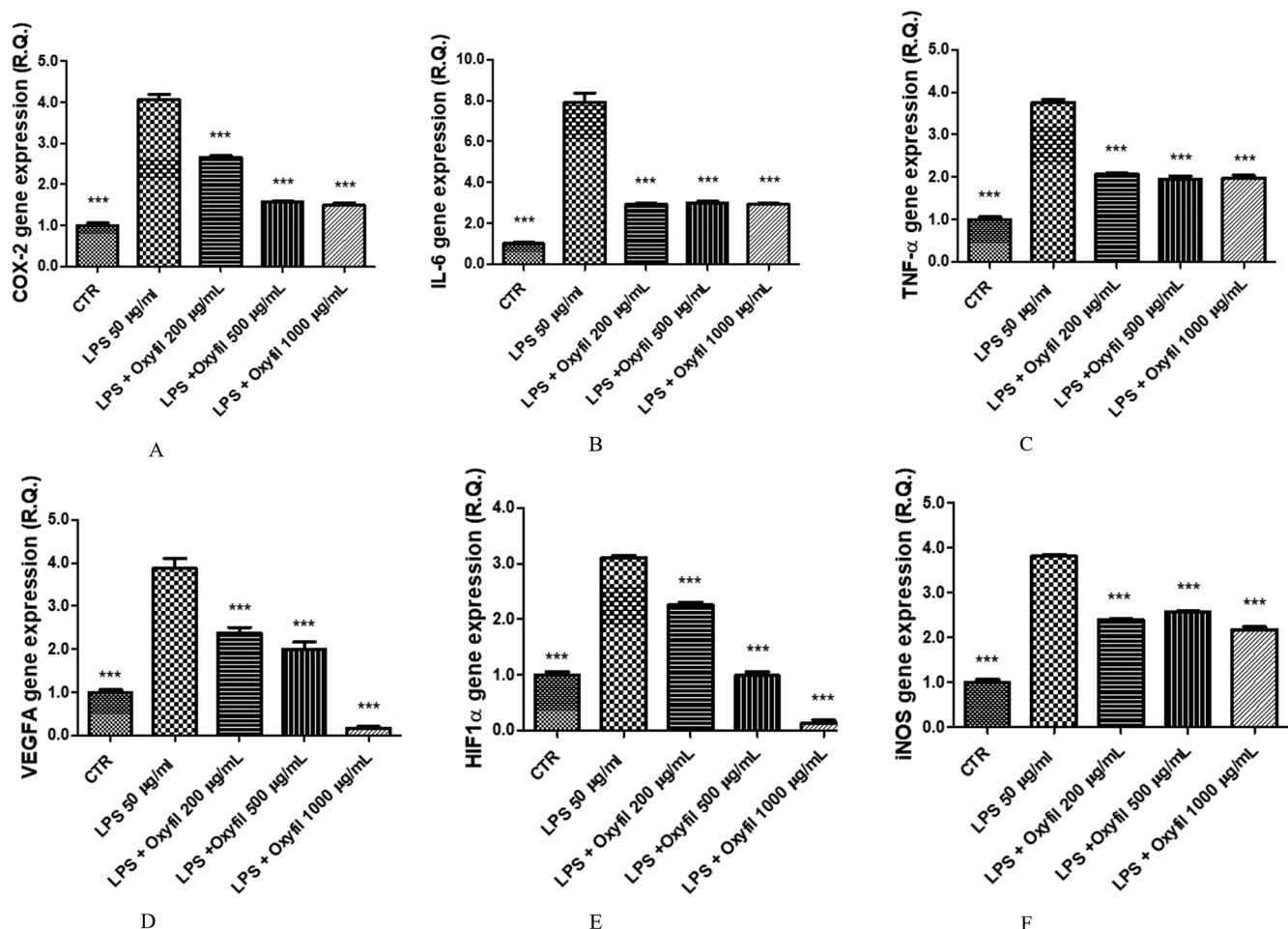


FIGURE 2 | (A) Inhibitory effects induced by the formulation Oxyfil (200–1000 µg/mL) on LPS-induced COX-2 gene expression (ANOVA, $p < 0.0001$, *** $p < 0.001$ vs. CTR). (B) Inhibitory effects induced by the formulation Oxyfil (200–1000 µg/mL) on LPS-induced IL-6 gene expression (ANOVA, $p < 0.0001$, *** $p < 0.001$ vs. CTR). (C) Inhibitory effects induced by the formulation Oxyfil (200–1000 µg/mL) on LPS-induced TNFα gene expression (ANOVA, $p < 0.0001$, *** $p < 0.001$ vs. CTR). (D) Inhibitory effects induced by the formulation Oxyfil (200–1000 µg/mL) on LPS-induced VEGFA gene expression (ANOVA, $p < 0.0001$, *** $p < 0.001$ vs. CTR). (E) Inhibitory effects induced by the formulation Oxyfil (200–1000 µg/mL) on LPS-induced HIF1α gene expression (ANOVA, $p < 0.0001$, *** $p < 0.001$ vs. CTR). (F) Inhibitory effects induced by the formulation Oxyfil (200–1000 µg/mL) on LPS-induced i-NOS gene expression (ANOVA, $p < 0.0001$, *** $p < 0.001$ vs. CTR).

TABLE 5 | Baseline characteristics of the study population by treatment group.

	Oxyfil (N=27)	Oxyfil + α -blocker (N=18)	Oxyfil + PDE5i (N=7)	Oxyfil +5- ARI (N=7)	Total (N=59)	p
Age (years)	64.0 (61.0, 67.5)	59.5 (56.2, 61.0)	50.0 (49.5, 50.5)	78.0 (69.5, 79.0)	61.0 (56.0, 65.0)	<0.001 (1)
BMI (kg/m ²)	24.0 (23.5, 24.5)	27.0 (25.0, 27.0)	26.0 (25.0, 26.5)	24.0 (22.0, 25.5)	24.0 (24.0, 27.0)	0.019 (1)
Hypertension	17 (63.0%)	10 (55.6%)	0 (0.0%)	1 (14.3%)	28 (47.5%)	0.006 (2)
Diabetes	1 (3.7%)	2 (11.1%)	0 (0.0%)	0 (0.0%)	3 (5.1%)	0.530 (2)
Cardiovascular disease	1 (3.7%)	4 (22.2%)	0 (0.0%)	1 (14.3%)	6 (10.2%)	0.170 (2)
History of Urinary Infections	0 (0.0%)	2 (11.1%)	1 (14.3%)	0 (0.0%)	3 (5.1%)	0.221 (2)
Prostate volume (mL)	40.0 (40.0, 45.0)	50.0 (46.2, 55.0)	30.0 (30.0, 35.0)	70.0 (70.0, 70.0)	45.0 (40.0, 55.0)	<0.001 (1)
Prostate adenoma volume (mL)	25.0 (25.0, 27.5)	30.0 (25.0, 35.0)	20.0 (20.0, 22.5)	40.0 (35.0, 40.0)	25.0 (25.0, 32.5)	<0.001 (1)

Note: Continuous variables are reported as median and interquartile range (IQR); categorical variables as counts and percentages. Comparisons among groups were performed using the Kruskal-Wallis test for continuous variables and Pearson's Chi-squared test for categorical variables.

TABLE 6 | Pre-post comparison of main outcomes following adjunction of Oxyfil to standard treatment.

	Pre-treatment (N=59)	Post-treatment (N=59)	Difference (N=59)	p
Q _{max}	13.0 (12.0, 14.5)	17.0 (16.0, 18.0)	3.0 (2.0, 4.0)	<0.001
PVR	80.0 (50.0, 85.0)	10.0 (10.0, 20.0)	-60.0 (-70.0, -40.0)	<0.001
IPSS	17.0 (12.0, 24.0)	8.0 (3.0, 9.0)	-7.0 (-18.0, -4.0)	<0.001
IIEF	17.0 (15.0, 18.0)	22.0 (22.0, 24.0)	5.0 (4.0, 9.0)	<0.001

Note: Continuous variables are reported as median and interquartile range (IQR). Differences between pre- and post-treatment values were assessed using the Wilcoxon signed-rank test for paired samples.

obstructive complaints. However, the non-randomized design, small cell sizes for some combinations (especially PDE5i), short follow-up, and absence of multiplicity correction reduce the inference strength.

Future studies should include a prospective randomized trial with stratification by baseline Q_{max} and prostate volume, pre-specified analysis of IPSS domains; monitoring adherence and adverse events would also enhance the interpretability of clinical outcomes.

Author Contributions

Silvia Villa: methodology, software, data curation, formal analysis, validation, investigation. **Annalisa Chiavaroli:** investigation, formal analysis, data curation, validation, software, methodology, resources, writing – original draft. **Gokhan Zengin:** software, data curation, visualization, writing – review and editing. **Silvia Messina:** methodology, software, data curation, investigation, formal analysis, validation. **Stefano Salvatore:** conceptualization, methodology, software, data curation, supervision. **Chiara Lupi:** methodology,

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The authors have nothing to report.

Conflicts of Interest

Michele Marchioni has received honoraria for advisory activities from Omega Pharma.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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