

Article

White Teff Flour Ethanolic Extract: Phytochemical Profile, Antioxidant and Anti-Inflammatory Activity

Ioana Ferentiu¹, Tiberia Ioana Pop^{2,*} , Alina Elena Pârvu¹ , Iulia Ioana Morar¹, Dinu Bolundut^{1,3} , Marcel Pârvu⁴ , Florica Ranga⁵, Ciprian Ovidiu Dalai⁶ , Mădălina Țicoalea¹ and Raluca Maria Pop⁷ 

- ¹ Pathophysiology, Department of Morphofunctional Sciences, Faculty of Medicine, “Iuliu Hațieganu” University of Medicine and Pharmacy Cluj-Napoca, 400012 Cluj-Napoca, Romania; abrudan.ferentiu.ioana@elearn.umfcluj.ro (I.F.); parvualinaelena@umfcluj.ro (A.E.P.); iulia.morar@umfcluj.ro (I.I.M.); bolundut_dinu@elearn.umfcluj.ro (D.B.); madalinaticolea@umfcluj.ro (M.Ț.)
 - ² Department of Technical and Soil Sciences, Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 400372 Cluj-Napoca, Romania
 - ³ Department of Medical Oncology, “Ion Chiricuță” Institute of Oncology, 400015 Cluj-Napoca, Romania
 - ⁴ Department of Biology, Babeș-Bolyai University, 400015 Cluj-Napoca, Romania; marcel.parvu@ubbcluj.ro
 - ⁵ Food Science and Technology, Department of Food Science, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 400372 Cluj-Napoca, Romania; florica.ranga@usamvcluj.ro
 - ⁶ Department of Dental Medicine, Faculty of Medicine and Pharmacy, University of Oradea, 410073 Oradea, Romania; cipridalai@gmail.com
 - ⁷ Pharmacology, Toxicology and Clinical Pharmacology, Department 2—Functional Sciences, Faculty of Medicine, “Iuliu Hațieganu” University of Medicine and Pharmacy Cluj-Napoca, 400012 Cluj-Napoca, Romania; raluca.pop@umfcluj.ro
- * Correspondence: tiberia.pop@usamvcluj.ro

Abstract

Teff (*Eragrostis tef*) is a gluten-free cereal increasingly promoted as a functional food, yet the bioactive profile and mechanistic evidence of some varieties remain limited. This study characterized an ethanolic extract of white teff flour and evaluated its antioxidant and anti-inflammatory potential *in vitro* and in a rat model of acute inflammation. White teff flour was extracted by cold repercolation (1 g/mL; 70% ethanol). Total polyphenols and flavonoids were quantified spectrophotometrically, and phenolics were profiled by HPLC-DAD-ESI-MS. Antioxidant activity was assessed using DPPH, FRAP, H₂O₂ scavenging, and NO scavenging assays. *In vivo*, acute inflammation was induced with intramuscular turpentine in Wistar rats, testing teff extract therapeutically (post-induction) and prophylactically (10-day pretreatment), with diclofenac and Trolox as comparators. Serum oxidative stress biomarkers (TOS, TAC, OSI, AOPP, MDA, NOx, 3-NT, thiols) and inflammatory mediators (NFκB-p65, IL-1β, IL-18, caspase-1, IL-10) were measured. The extract showed low total polyphenols (0.044 ± 0.002 mg GAE/g d.w.) and higher flavonoids (11.83 ± 1.10 mg QE/100 g d.w.). Eighteen phenolics were identified (total 398.30 ± 1.48 μg/mL), dominated by flavone derivatives (notably apigenin- and luteolin-glycosides), while phenolic acids accounted for ~33.21%. *In vitro* antioxidant capacity was robust (DPPH 286.17 ± 11.52 μg TE/g d.w.; FRAP 263.17 ± 20.09 μg TE/g d.w.; H₂O₂ 214.12 ± 18.22 mg TE/g d.w.; NO 300.77 ± 28.71 mg QE/g d.w.). *In vivo*, turpentine provoked marked oxidative stress and inflammatory activation; teff, particularly at the highest concentration and in prophylaxis, reduced nitro-oxidative damage markers (AOPP, MDA, NOx, 3-NT) and lowered NFκB-p65, IL-1β, IL-18, and caspase-1, while IL-10 was not significantly altered. White teff flour ethanolic extract contains a flavone-rich phenolic profile and exerts measurable antioxidant and anti-inflammatory effects in an acute inflammation model, supporting its potential development as a nutraceutical candidate.



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Keywords: *Eragrostis tef*; phenolic compounds; flavone glycosides; antioxidant activity; anti-inflammatory effects

1. Introduction

Teff (*Eragrostis tef*) is a cereal originally domesticated in Ethiopia [1]. It belongs to the *Poaceae* family (the grass family), within the genus *Eragrostis*, which includes roughly 350 species; teff is the only species widely cultivated for food [2–4]. Beyond Ethiopia, cultivation has expanded to multiple regions, including Australia, parts of Africa, North America, and Europe [1]. Recent work also suggests that teff can be grown under Mediterranean climatic conditions [4,5]. In Ethiopian cuisine, teff flour is traditionally used to prepare injera, a fermented flatbread [6].

Teff is commonly marketed as white, brown, or mixed varieties [7]. Because teff flour is typically milled as a whole grain, it retains bran-derived components [8]. Multiple studies report that teff is naturally gluten-free, and provides slowly digestible carbohydrates (often with a low glycemic index), dietary fiber, essential amino acids, vitamins, minerals, and diverse polyphenols [7–10]. Teff’s nutritional value is superior compared to that of other gluten-free grains, like quinoa and rice, with teff being richer in fiber, iron, and calcium than flours and other gluten-free products [11]. Consequently, teff has been proposed as a functional food/nutraceutical in a range of chronic conditions, and it is a suitable staple for individuals requiring lifelong gluten avoidance, including patients with celiac disease [12–15].

The World Health Organization describes functional foods as natural foods that contain bioactive components (including phytochemicals) that may contribute to the prevention and management of chronic non-communicable diseases [12,16]. The term nutraceutical (from “nutrition” and “pharmaceutical”) refers to naturally derived products obtained from plant- or animal-based food that may exert pharmacologically relevant effects [17]. In the context of inflammation, functional foods and nutraceuticals are typically expected to provide antioxidant and/or anti-inflammatory activity [12,16], and current research focuses on their mechanisms of action, safety profiles, and clinical evidence [18].

Given the growing interest in teff as a nutrient-dense gluten-free grain [1,9] and the limited characterization of some varieties [3,19], this study evaluated the bioactive profile of a white teff flour ethanolic extract. We further aimed to generate evidence supporting the development of a nutraceutical based on white teff extract by assessing its antioxidant and anti-inflammatory mechanisms in an experimental model of inflammation.

2. Results

2.1. Phytochemical Analysis

The white teff ethanolic extract exhibited a relatively low total polyphenol content, while total flavonoids were comparatively higher (Table 1).

Table 1. Total polyphenol content and total flavonoid content of white teff ethanolic extract.

Plant Extract	Total Polyphenol Content (mg GAE/g d.w. Plant Material)	Total Flavonoid Content (µg QE/g d.w. Plant Material)
White teff 1 g/1 mL	0.044 ± 0.002	11.83 ± 1.10

GAE—gallic acid equivalents; QE—quercetin equivalents.

HPLC-DAD-ESI-MS profiling revealed appreciable levels of phenolic acids and flavones. Eighteen compounds were tentatively identified based on retention time, UV

spectra, and MS signals. Phenolic acids included three hydroxybenzoic acid derivatives (2,3-dihydroxybenzoic acid, 2-hydroxybenzoic acid, protocatechuic acid) and one hydroxycinnamic acid (chlorogenic acid), together accounting for approximately 33.21% of the total identified phenolics. Most remaining peaks corresponded to flavones; apigenin derivatives represented 16.62% and luteolin derivatives 16.59% of the identified profile (Figure 1; Table 2).

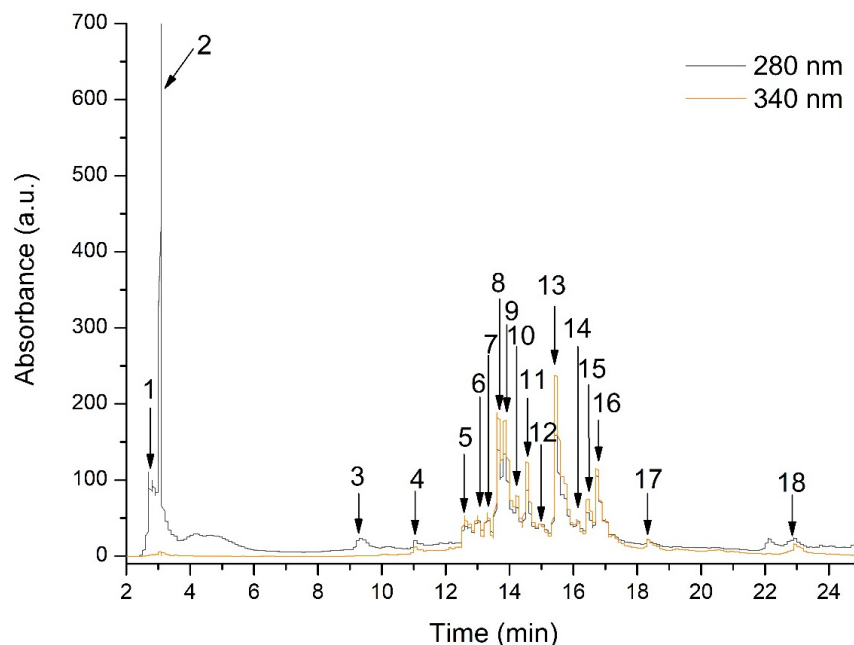


Figure 1. HPLC chromatogram of phenolic compounds from white teff ethanolic extract measured at 280 and 340 nm. The peak identification is provided in Table 2.

Table 2. Liquid Chromatography–Diode Array Detection–Electrospray Ionization Mass Spectrometry for phenolic-compound tentative identification from white teff ethanolic extract.

Peak No.	R _t (min)	UV λ _{max} (nm)	[M + H] ⁺ (m/z)	Compounds	Concentration (μg/mL)
1	2.71	280	139	2-Hydroxybenzoic acid ¹	20.16 ± 0.05
2	3.07	275	155	2,3-Dihydroxybenzoic acid ¹	101.06 ± 0.51
3	9.33	280	155	Protocatechuic acid ¹	11.09 ± 0.09
4	11.07	330	355	5-Caffeoylquinic acid (Chlorogenic acid) ²	6.06 ± 0.27
5	12.59	350.270	611.287	Luteolin-diglucoside ³	6.68 ± 0.08
6	12.99	350.270	595.287	Luteolin-rutinoside ³	9.12 ± 0.29
7	13.29	350.270	433.287	Luteolin-rhamnoside ³	9.74 ± 0.19
8	13.61	340.270	595.271	Apigenin-diglucoside ³	25.21 ± 0.08
9	13.85	350.270	757.287	Luteolin-glucosyl-neohesperidoside ³	40.57 ± 1.33
10	14.22	340.270	565.271	Apigenin-arabinosyl-galactoside ³	13.73 ± 0.26
11	14.53	350.270	449.287	Luteolin-glucoside ³	25.99 ± 0.10
12	14.93	340.270	565.271	Apigenin-arabinosyl-glucoside ³	11.38 ± 0.09
13	15.44	340.270	433.271	Apigenin-glucoside ³	64.71 ± 0.11
14	16.11	340.270	447.271	Apigenin-methylglucoside ³	9.42 ± 0.15
15	16.45	340.270	775.271	Apigenin-syringyl-diglucoside ³	13.27 ± 0.13
16	16.74	340.270	579.271	Apigenin-neohesperidoside ³	19.79 ± 0.92
17	18.34	340.270	475.271	Apigenin-acetyl-glucoside ³	5.86 ± 0.32
18	22.93	340.270	271	Apigenin ³	4.23 ± 0.11
				Total phenolics	398.30 ± 1.48

¹—compounds belonging to hydroxybenzoic acid subclass; ²—compounds belonging to hydroxycinnamic acid subclass; ³—compounds belonging to flavone subclass.

In vitro antioxidant capacity, assessed by DPPH, FRAP, H₂O₂ scavenging, and NO scavenging assays, indicated robust activity for teff. Compared with Trolox (TX), TEFF showed stronger effects in the DPPH, FRAP, and H₂O₂ assays ($p < 0.001$). NO scavenging was also significant relative to quercetin ($p < 0.001$) (Table 3).

Table 3. *In vitro* antioxidant activity of the white teff ethanolic extract.

Sample	DPPH ($\mu\text{g TE/g d.w. Plant Material}$)	FRAP ($\mu\text{g TE/g d.w. Plant Material}$)	H ₂ O ₂ Scavenging Activity ($\text{mg TE/g d.w. Plant Material}$)	NO Scavenging Activity ($\text{mg QE/g d.w. Plant Material}$)
White teff extract (1 gr/1 mL)	286.17 $\pm$ 11.52	263.17 $\pm$ 20.09	214.12 $\pm$ 18.22	300.77 $\pm$ 28.71
Trolox IC50	12.01 $\pm$ 1.6	13.21 $\pm$ 1.81	22.19 $\pm$ 2.06	-
Quercetin IC50			-	19.59 $\pm$ 2.32
<i>p</i> -value	0.001	0.001	0.001	0.001

DPPH—DPPH free-radical scavenging capacity; FRAP—ferric reducing antioxidant power; H₂O₂—hydrogen peroxide scavenging capacity; NO—nitric oxide radical scavenging assay; TE—Trolox equivalent; QE—quercetin equivalent.

2.2. *In Vivo* Antioxidant Activity of the White Teff Ethanolic Extract

Systemic redox status was evaluated by measuring oxidant markers (TOS, OSI, AOPP, MDA, NOx, 3-NT) and antioxidant markers (TAC, total thiols, SH) (Table 4).

Compared with SHAM, the INFL group exhibited a pronounced pro-oxidative shift, reflected by increased TOS and OSI (both $p < 0.001$) and reduced TAC ($p < 0.001$). This was accompanied by elevated AOPP, MDA, NOx, and 3-NT (all $p < 0.001$), and a significant decrease in SH ($p < 0.001$) (Table 4).

Diclofenac attenuated oxidative stress, with reductions in TOS ($p < 0.05$) and OSI ($p < 0.01$) and a marked increase in TAC ($p < 0.001$). AOPP ($p < 0.001$), MDA ($p < 0.01$), NOx ($p < 0.01$), and 3-NT ($p < 0.001$) were also lowered relative to INFL (Table 4).

Trolox (TX) decreased OSI ($p < 0.05$), driven by a modest reduction in TOS ($p < 0.05$) and a significant increase in TAC ($p < 0.001$). Oxidative damage markers (AOPP, MDA, NOx, and 3-NT) decreased (p -values as shown in Table 4), and TAC elevation was paralleled by increased SH ($p < 0.001$). Overall, no significant differences were detected between diclofenac and Trolox in their effects on oxidative stress (Table 4).

After inflammation induction, a clear reduction in TOS and OSI was observed primarily in INFL/TEFF100 ($p < 0.01$). TAC increased moderately across teff-treated groups ($p < 0.05$). Among oxidant markers, MDA was not significantly affected, whereas NOx, 3-NT, and AOPP decreased in a dose-dependent manner, with the highest teff concentration showing the strongest effect ($p < 0.001$) (Table 4). SH did not change significantly with TEFF treatment ($p > 0.05$).

In the prophylactic protocol, TEFF100/INFL and TEFF50/INFL prevented the turpentine-induced rise in TOS and OSI ($p < 0.001$), and all teff dilutions increased TAC ($p < 0.001$). TOS reduction coincided with substantial decreases in AOPP and 3-NT across all prophylactic groups ($p < 0.001$). A smaller decrease in MDA and NOx was noted mainly in TEFF100/INFL ($p < 0.01$). SH increased significantly in TEFF50/INFL and TEFF25/INFL ($p < 0.01$) (Table 4).

Table 4. *In vivo* antioxidant activity of the white teff ethanolic extract.

Groups	TOS (μmol H_2O_2 Equiv./L)	TAC (mmol Trolox Equiv./L)	OSI	AOPP ($\mu\text{mol/L}$)	MDA (nmol/L)	NOx ($\mu\text{mol/L}$)	3-NT (ng/mL)	SH ($\mu\text{mol/L}$)
CONTROL	5.70 $\pm$ 1.10 ***	1.09 $\pm$ 0.00 ***	5.50 $\pm$ 1.04 ***	81.36 $\pm$ 15.17 ***	4.89 $\pm$ 0.41 ***	30.52 $\pm$ 3.96 ***	26.00 $\pm$ 2.84 ***	582.00 $\pm$ 10.97 ***
INFL	16.16 $\pm$ 1.84	1.08 $\pm$ 0.00	16.23 $\pm$ 1.85	140.51 $\pm$ 14.80	6.83 $\pm$ 0.44	79.79 $\pm$ 2.79	55.95 $\pm$ 3.48	317.40 $\pm$ 34.70
INFL/DICLO	9.62 $\pm$ 1.65 *	1.09 $\pm$ 0.00 ***	8.57 $\pm$ 1.59 **	95.22 $\pm$ 10.23 **	5.12 $\pm$ 0.81 *	51.78 $\pm$ 2.06 **	22.89 $\pm$ 2.92 ***	373.40 $\pm$ 25.08
INFL/TX	8.36 $\pm$ 1.09 *	1.09 $\pm$ 0.00 ***	8.55 $\pm$ 1.16 **	92.78 $\pm$ 9.87 **	5.16 $\pm$ 0.18 *	54.21 $\pm$ 8.70 **	23.68 $\pm$ 4.16 ***	641.33 $\pm$ 54.06 ***
INFL/TEFF100	8.21 $\pm$ 1.78 **	1.09 $\pm$ 0.00 *	7.47 $\pm$ 1.82 **	57.65 $\pm$ 6.11 ***	6.00 $\pm$ 0.23	25.11 $\pm$ 1.96 ***	22.34 $\pm$ 3.42 ***	374.33 $\pm$ 13.00
INFL/TEFF50	12.50 $\pm$ 2.57	1.09 $\pm$ 0.00 *	12.49 $\pm$ 1.88	89.41 $\pm$ 2.95 **	6.57 $\pm$ 0.38	29.45 $\pm$ 2.85 **	25.18 $\pm$ 3.64 **	358.33 $\pm$ 31.77
INFL/TEFF25	14.55 $\pm$ 2.66	1.09 $\pm$ 0.00 *	12.95 $\pm$ 1.63	107.51 $\pm$ 7.07 *	6.56 $\pm$ 0.12	56.83 $\pm$ 4.07 **	32.07 $\pm$ 4.25 *	346.33 $\pm$ 26.39
TEFF100/INFL	7.66 $\pm$ 1.53 ***	1.09 $\pm$ 0.00 ***	6.94 $\pm$ 1.62 **	66.08 $\pm$ 4.77 ***	5.80 $\pm$ 0.13 *	55.35 $\pm$ 2.57 **	21.06 $\pm$ 1.16 ***	398.60 $\pm$ 19.74
TEFF50/INFL	9.63 $\pm$ 2.86 ***	1.09 $\pm$ 0.00 ***	8.59 $\pm$ 1.84 **	68.55 $\pm$ 3.45 ***	6.15 $\pm$ 0.31	70.70 $\pm$ 5.51	18.27 $\pm$ 1.68 ***	496.67 $\pm$ 22.66 **
TEFF25/INFL	13.76 $\pm$ 2.55	1.09 $\pm$ 0.00 ***	13.55 $\pm$ 2.18	70.43 $\pm$ 5.95 ***	6.19 $\pm$ 0.30	72.98 $\pm$ 6.99	11.85 $\pm$ 1.22 ***	509.67 $\pm$ 39.45 ***

INFL—inflammation; DICLO—diclofenac; TX—Trolox; TEFF100—white teff 100%; TEFF50—white teff 50%; TEFF25—white teff 25%; TOS—total oxidative status; TAC—total antioxidant capacity; OSI—oxidative stress index; AOPP—advanced oxidation protein products; MDA—malondialdehyde; NOx—nitrites and nitrates; 3-NT—3-nitrotyrosine; SH—total thiols. Vs INFL: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Principal component analysis (PCA) was performed to investigate the relationships between oxidative stress and inflammatory biomarkers and to evaluate the global effect of therapeutic interventions. Because the correlation matrix was not positive definite, reflecting strong collinearity between OSI and its constituent parameters (TOS and TAC), PCA was interpreted using an oblique rotation (Direct Oblimin). Communalities after extraction were generally high (range 0.469–0.988), indicating that most variables were well represented by the retained components. Four principal components were retained, together explaining 80.0% of the total variance (PC1 = 43.3%, PC2 = 16.4%, PC3 = 11.4%, PC4 = 9.0%).

The rotated structure showed that PC1 captured a major oxidative–inflammatory axis, with high positive loadings for IL-1 β , MDA, NF- κ B, IL-18, 3-NT, and NO and negative loadings for SH and TAC, reflecting concomitant oxidative damage and antioxidant depletion. PC2 was mainly associated with AOPP, NO, and caspase activity, indicating protein oxidation and apoptosis-related processes. PC3 was defined primarily by IL-10, suggesting regulatory or counter-inflammatory signaling, while PC4 was dominated by TOS and OSI, representing global oxidant status. Overall, PCA demonstrates that therapeutic administration modulates several interrelated but partially independent oxidative and inflammatory pathways rather than a single biological dimension (Figure 2).

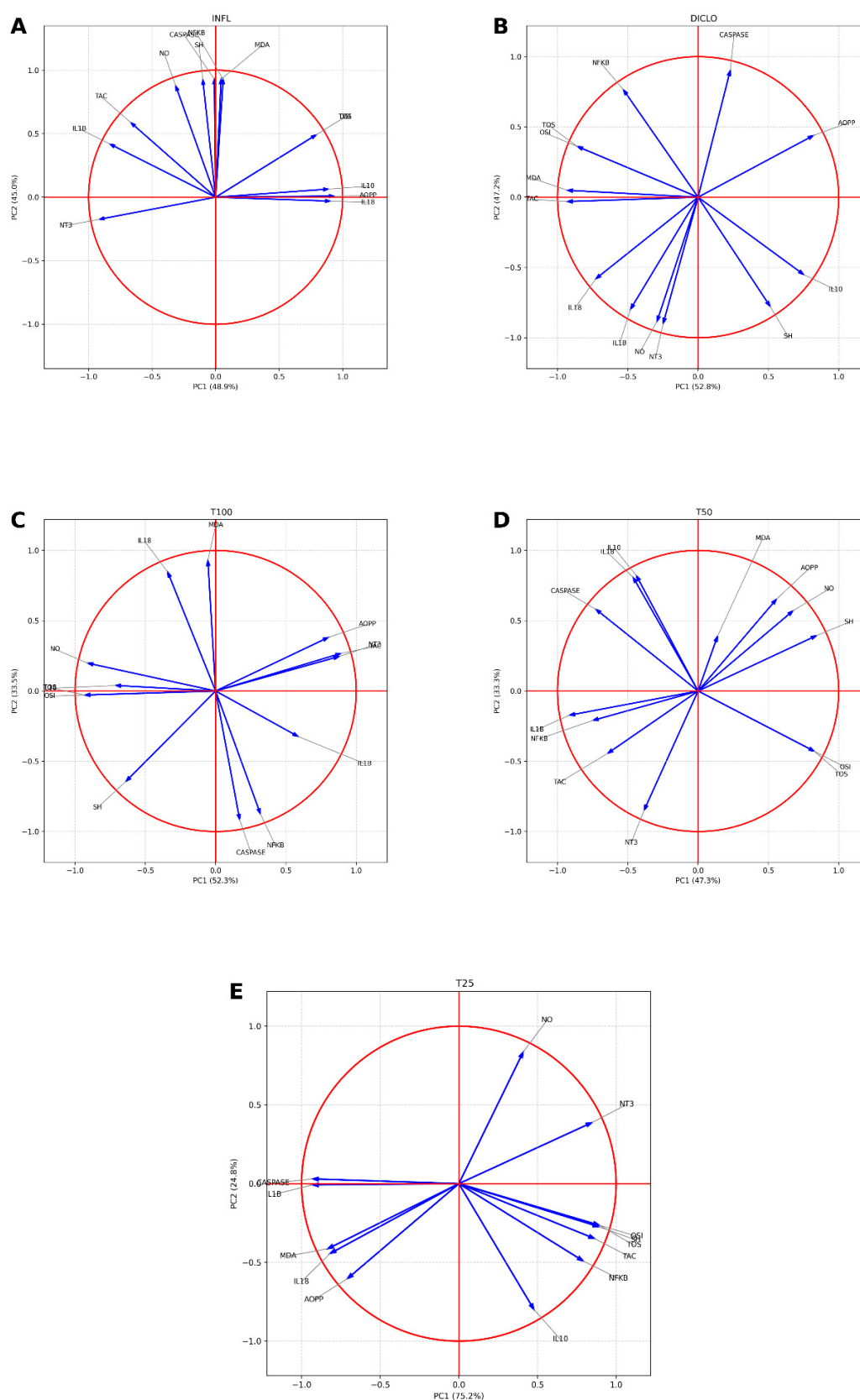


Figure 2. PCA correlation circles of oxidative stress and inflammatory markers in experimental groups. Vectors represent the contribution and correlation of each biomarker with the first two principal components (PC1 and PC2). (A) INFL group; (B) DICLO group; (C) T100% group; (D) T50% group; (E) T25% group. The direction and length of vectors indicate the weight of each variable in discriminating between groups.

2.3. In Vivo Inflammatory Markers

Inflammatory response was assessed by measuring NFkB-p65, IL-1 β , IL-18, and caspase-1 (as indirect markers of NLRP3 inflammasome activity), together with IL-10 as an anti-inflammatory cytokine. Relative to SHAM, INFL animals showed significant increases in NFkB-p65, IL-1 β , IL-18, and caspase-1 ($p < 0.01$), while IL-10 did not change significantly ($p > 0.05$) (Table 5).

Compared with INFL, diclofenac reduced NFkB-p65, IL-1 β , IL-18, and caspase-1 (all $p < 0.001$). Trolox also reduced NFkB-p65 ($p < 0.05$), IL-1 β ($p < 0.001$), IL-18 ($p < 0.001$), and caspase-1 ($p < 0.05$) (Table 5).

Post-induction TEFF treatment significantly lowered NFkB-p65, IL-1 β , IL-18, and caspase-1 ($p < 0.001$). Relative to diclofenac, TEFF showed a weaker reduction in NFkB-p65, IL-1 β , and caspase-1, but a stronger reduction in IL-18. Compared with Trolox, TEFF had smaller effects on NFkB-p65, IL-1 β , and IL-18, while exhibiting a stronger reduction in caspase-1 (Table 5).

Pre-treatment with teff for 10 days mitigated the turpentine-associated rise in NFkB-p65 ($p < 0.001$), IL-1 β ($p < 0.01$), IL-18 ($p < 0.001$), and caspase-1 ($p < 0.001$). Overall, prophylactic administration tended to produce stronger inhibition of inflammatory markers than therapeutic administration. Aside from caspase-1, TEFF100 and TEFF50 showed a more pronounced preventive effect than TEFF25; for the other markers, the three dilutions showed comparable efficacy (Table 5).

IL-10 levels were not significantly influenced by any of the tested interventions ($p > 0.05$) (Table 5).

Table 5. *In vivo* anti-inflammatory activity of the white teff ethanolic extract.

Groups	Nfkb-p65 (pg/mL)	IL-1b (pg/mL)	IL-18 (pg/mL)	Caspase-1 (pg/mL)	IL-10 (pg/mL)
CONTROL	139.72 $\pm$ 25.92 ***	26.37 $\pm$ 1.85 ***	8.03 $\pm$ 1.14 ***	51.40 $\pm$ 3.63 ***	61.24 $\pm$ 4.44
INFL	589.83 $\pm$ 63.19	64.52 $\pm$ 2.70	22.61 $\pm$ 2.84	252.07 $\pm$ 15.69	65.21 $\pm$ 5.55
INFL/DICLO	157.73 $\pm$ 35.19 ***	33.88 $\pm$ 2.29 ***	12.95 $\pm$ 2.46 ***	12.85 $\pm$ 1.90 ***	65.65 $\pm$ 2.99
INFL/TX	155.06 $\pm$ 34.42 ***	34.97 $\pm$ 4.29 ***	8.52 $\pm$ 2.17 ***	65.09 $\pm$ 8.83 ***	63.74 $\pm$ 4.46
INFL/TEFF100	203.70 $\pm$ 66.20 ***	52.59 $\pm$ 5.29 **	8.98 $\pm$ 2.41 ***	32.64 $\pm$ 4.19 ***	66.82 $\pm$ 3.99
INFL/TEFF50	255.09 $\pm$ 35.52 ***	45.26 $\pm$ 3.83 **	9.59 $\pm$ 1.27 ***	31.72 $\pm$ 2.75 ***	62.71 $\pm$ 2.99
INFL/TEFF25	178.55 $\pm$ 27.31 ***	45.97 $\pm$ 4.82 **	8.86 $\pm$ 2.47 ***	38.60 $\pm$ 3.50 ***	65.94 $\pm$ 2.17
TEFF100/INFL	137.68 $\pm$ 24.52 ***	44.41 $\pm$ 8.32 **	7.24 $\pm$ 1.75 ***	25.25 $\pm$ 4.05 ***	64.47 $\pm$ 1.56
TEFF50/INFL	126.53 $\pm$ 8.42 ***	46.15 $\pm$ 5.37 **	7.50 $\pm$ 0.70 ***	58.49 $\pm$ 3.18 ***	64.91 $\pm$ 5.34
TEFF25/INFL	170.46 $\pm$ 53.10 ***	41.61 $\pm$ 5.31 **	8.30 $\pm$ 1.69 ***	125.19 $\pm$ 16.72 **	65.35 $\pm$ 9.72

INFL—inflammation; DICLO—diclofenac; TX—Trolox; TEFF100—white teff 100%; TEFF50—white teff 50%; TEFF25—white teff 25%; Nfkb-p65—nuclear factor- κ B; IL-1b—interleukine 1-b; IL-18—interleukine 18; IL-18—interleukine 10; ** $p < 0.01$; *** $p < 0.001$.

Principal component analysis (PCA) was performed to explore the multivariate relationships among oxidative stress and inflammatory biomarkers following prophylactic administration. Based on eigenvalues > 1 and inspection of the scree plot, three principal components were retained, together explaining 76.7% of the total variance (PC1: 52.8%, PC2: 13.5%, PC3: 10.5%). Communalities after extraction were generally high, indicating adequate representation of most variables within the retained component space. The rotated solution revealed that PC1 was characterized by high positive loadings of NF- κ B, caspase activity, OSI/TOS, MDA, IL-1 β , IL-18, NO, and 3-NT, together with negative loadings for SH and TAC, reflecting a dominant axis integrating oxidative stress burden, inflammatory signaling, and reduced antioxidant capacity. PC2 was primarily associated with AOPP and NO, indicating variability related to protein oxidation and nitric oxide-dependent processes, while PC3 was mainly defined by IL-10, representing an independent regulatory inflammatory dimension. Correlations between components were low, confirming that

these axes describe largely distinct biological patterns. Specific correlation circles illustrated a progressive redistribution of biomarkers across the PCA space. The INFL group showed tight clustering of oxidative and inflammatory markers along PC1. In contrast, the T100 group displayed a marked displacement of several pro-oxidant and inflammatory variables away from PC1, with relative preservation of antioxidant-related parameters. The T50 group exhibited an intermediate configuration, whereas the T25 group remained closer to the inflamed profile. Overall, PCA demonstrates a dose-dependent modulation of the oxidative–inflammatory biomarker network following therapeutic intervention (Figure 3).

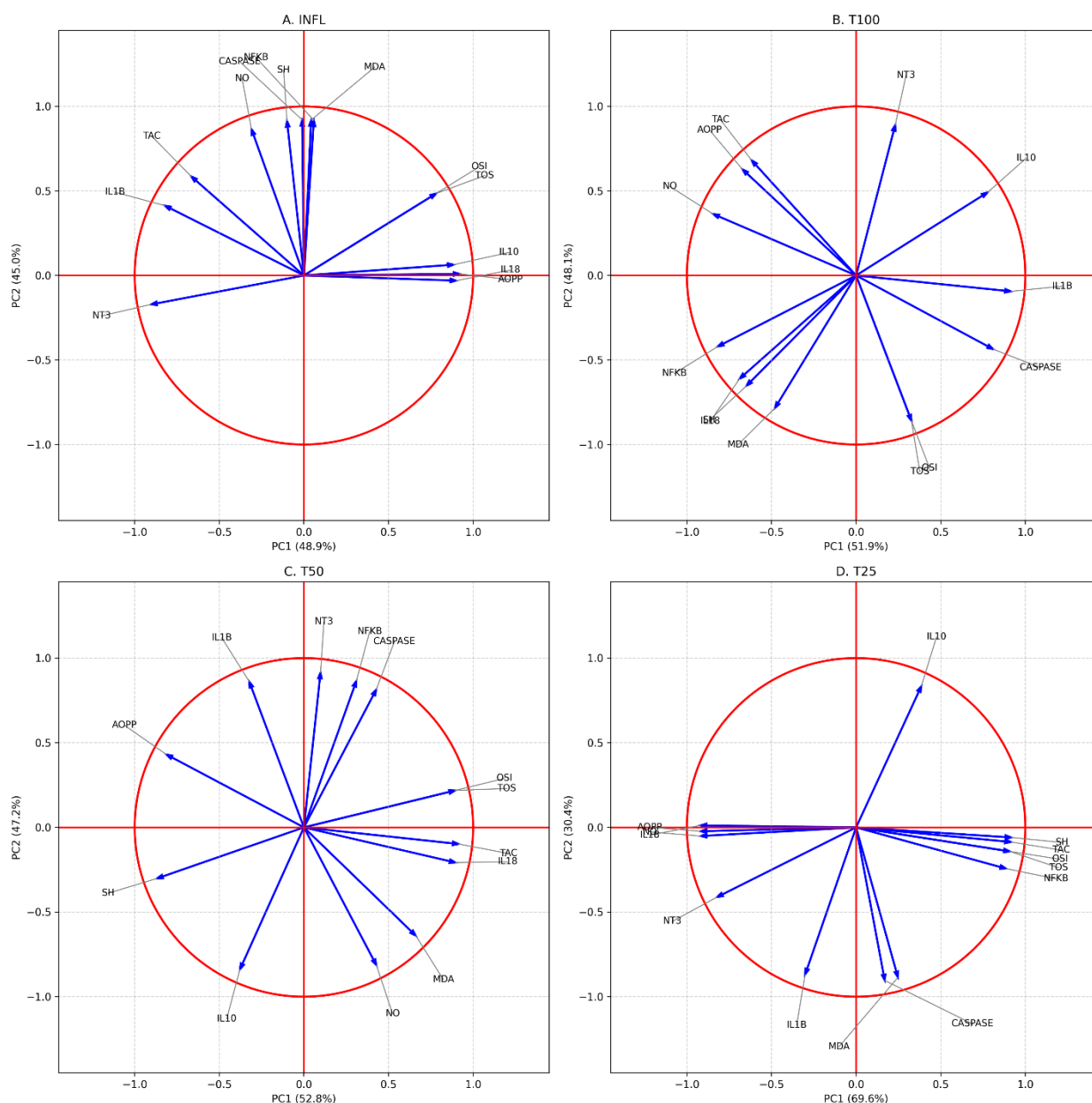


Figure 3. PCA correlation circles of oxidative stress and inflammatory markers in the prophylactic experimental setting. Vectors represent the contribution and correlation of each biomarker with the first two principal components (PC1 and PC2). (A) Inflammation group (INFL); (B) TEFF extract 100% group (T100); (C) TEFF extract 50% group (T50); and (D) TEFF extract 25% group (T25). The orientation and length of the vectors indicate the degree of association of each variable with the principal components and their relative contribution to group discrimination.

3. Discussion

Teff (*Eragrostis tef*) is a long-cultivated cereal from the Horn of Africa that has recently gained international visibility because of its nutritional quality and potential health benefits. Teff is naturally gluten-free [3] and provides protein, dietary fiber, and micronutrients such as iron, calcium, zinc, and magnesium [4,7,17]. Beyond basic nutrition, teff contains bioactive constituents (e.g., polyphenols, flavonoids, phytosterols, and saponins) that have been associated with antioxidant and anti-inflammatory effects [4,20]. Although teff has a long history of cultivation, systematic characterization of its phytochemical profile has expanded mainly in recent years [4].

Polyphenols comprise a diverse group of plant secondary metabolites, and many are recognized for their capacity to counter oxidative and inflammatory processes. Flavonoids represent an important polyphenol subclass and are present in teff. Both preclinical and clinical evidence suggests that sustained intake of polyphenol-rich diets may be associated with lower risk or improved management of several chronic conditions, including cardiovascular and neurodegenerative diseases, diabetes, cancer, and inflammatory disorders [18].

The phenolic profile of teff is influenced by cultivar and genetics, agronomic practices, environmental conditions, and extraction methodology. Reports for methanolic teff extracts describe TPC values of 46–133 mg GAE/100 g and TFC values of 15–113 mg QE/100 g, with brown varieties often exhibiting higher phenolics than white varieties, due to observable variations in the agroecological zones and the genetic make-up of the samples [1,21]. In the present work, ethanol-extracted TPC and TFC were lower than some values reported for methanol or ultrasound-assisted extraction [8,22]. When compared with other grains, like white fonio seeds, teff had a higher TPC [23].

In our HPLC-DAD-ESI+ analysis, white teff flour extract displayed a broad range of phenolic constituents. This is consistent with earlier reports showing that teff flours can contain higher phenolic levels than other refined cereal flours, and that soluble versus bound phenolics may differ between white and brown teff varieties [1,24,25].

Phenolic acids (e.g., ferulic, caffeic, and p-coumaric acids) and flavonoids (e.g., luteolin, apigenin, quercetin, rutin) have been described in teff, with substantial amounts located in the bran fraction [4,12,22]. These compounds can contribute to antioxidant capacity through radical scavenging, metal chelation, and modulation of enzyme activity [12].

In the present ethanolic extract, we detected hydroxybenzoic acid derivatives (2,3-dihydroxybenzoic acid, 2-hydroxybenzoic acid, and protocatechuic acid) together with the hydroxycinnamic acid chlorogenic acid. Such phenolic acids are frequently linked to antioxidant activity and have also been discussed in the context of anti-inflammatory, antimicrobial, and anticancer effects [18]. The dominant phenolics in our profile were flavones, mainly glycosylated derivatives of luteolin and apigenin. These flavonoids have been reported to interfere with NF- κ B signaling and other pro-inflammatory pathways, thereby limiting cytokine release [4]. Luteolin, widely distributed in plants, has been associated with Nrf2-related antioxidant responses and NF- κ B-related inflammatory responses [26], as well as metabolic effects in models of insulin resistance and diabetic complications [27,28]. In our extract, a luteolin-glucosyl-arabinoside derivative was the most abundant luteolin-related compound. Among apigenin derivatives, apigenin-glucoside and apigenin-diglucoside predominated. Apigenin has been discussed as a potential antidiabetic agent through mechanisms including stimulation of insulin secretion, inhibition of α -glucosidase, and ROS scavenging [8].

Oxidative stress reflects an imbalance between pro-oxidant species and antioxidant defenses, which can promote cellular injury and sustain inflammatory signaling. Persistent oxidative stress has been implicated in the pathogenesis of cardiovascular disease, neurodegeneration, diabetes, and malignancy. Antioxidants mitigate oxidative damage

by neutralizing reactive species. They include endogenous systems (e.g., enzyme-based defenses) and exogenous antioxidants obtained from one's diet. Whole grains, fruits, and vegetables are important dietary contributors to exogenous antioxidant intake.

To characterize bioactivity, nutraceutical candidates are commonly evaluated *in vitro* and *in vivo* [18]. Although polyphenols are widely studied as antioxidants, under certain *in vivo* conditions they may also exhibit pro-oxidant behavior. This shift can depend on factors such as solubility, metal chelation/reduction properties, and local pH [29]. Accordingly, we first assessed the antioxidant potential of white teff extract using DPPH, FRAP, H₂O₂, and NO scavenging assays.

The DPPH and FRAP results supported notable antioxidant capacity, consistent with the presence of multiple phenolic compounds. However, total phenolics (TPC/TFC) did not show a strong linear relationship with radical scavenging activity, which is plausible because antioxidant performance depends on both concentration and the chemical structures of individual phenolics. Previous work comparing white and brown teff has reported measurable levels of phenolics and flavonoids as well as antioxidant activity in both free and bound fractions. For example, there were reported flavonoids in the range of 0.52–1.02 mg RE/g and total phenolics of 0.90–1.42 mg GAE/g, with higher antioxidant activity in the free phenolic fraction (ABTS: 1.70–4.37 mmol TEAC/g) [21]. Other studies also report higher FRAP values in red/brown teff compared with white teff, likely reflecting differences in phenolic profiles [22,30].

H₂O₂ and NO scavenging assays further indicated that white teff extract can neutralize specific oxidants/reactive species. These observations suggest a potential role for teff-derived compounds in limiting nitro-oxidative stress, a process associated with aging and chronic disease development.

Oxidative stress arises when reactive oxygen species (ROS) generation exceeds the capacity of antioxidant defenses. ROS include species such as superoxide, hydrogen peroxide, hydroxyl radicals, and peroxynitrite, which can damage lipids, proteins, and nucleic acids when present at high levels [31].

Organisms counter ROS and reactive nitrogen species through coordinated enzymatic defenses (e.g., superoxide dismutase, catalase, peroxidases) and non-enzymatic antioxidants (e.g., glutathione, ascorbate, thiol groups, and plasma proteins) [32]. Because oxidative injury can manifest across multiple biomolecule classes, combined assessment of oxidant and antioxidant markers can provide a more robust view of redox status [33].

Although polyphenols are widely studied as antioxidants, under certain *in vivo* conditions they may also exhibit pro-oxidant behavior. This shift can depend on factors such as solubility, metal chelation/reduction properties, and local pH [29].

Given the complexity of oxidative stress, using a panel of oxidant and antioxidant biomarkers is considered a sound approach.

TOS provides an integrated estimate of circulating oxidants, whereas TAC captures the cumulative antioxidant capacity of a sample. Interpreting these together is informative: lower TOS and/or higher TAC suggests a more favorable redox balance. OSI, calculated from TOS and TAC, summarizes this balance as a single index [29,34]. In our model, turpentine-induced inflammation produced an oxidant shift (increased TOS/OSI and reduced TAC). Both diclofenac and Trolox improved these parameters, supporting the link between inflammatory signaling and redox imbalance. White teff extract also reduced oxidative stress in both therapeutic and prophylactic protocols, with a dose-dependent pattern and the undiluted extract (TEFF100) performing comparably to diclofenac and Trolox.

To explore mechanisms beyond global indices, we examined protein and lipid oxidation markers. Because many ROS are short-lived, downstream oxidation products in proteins and lipids can serve as practical indicators of oxidative injury [35]. AOPP are

oxidatively modified proteins (often albumin) generated by chlorinated oxidants such as hypochlorous acid produced during neutrophil activation [35,36]. AOPP may also propagate inflammation by promoting cytokine release and endothelial dysfunction, creating a reinforcing loop between oxidative stress and inflammation [37]. In our study, Trolox lowered AOPP, and white teff extract reduced AOPP even more markedly than diclofenac and Trolox in both experimental schemes.

MDA, a widely used lipid peroxidation marker, reflects oxidative degradation of polyunsaturated fatty acids and is often used to assess dietary or supplement effects on membrane lipid damage [38]. Teff polyphenols have been discussed as inhibitors of lipid peroxidation [12]. In our experiments, white teff extract reduced MDA after and before inflammation induction in a dose-dependent manner, although the magnitude of effect was smaller than with diclofenac or Trolox.

Nitric oxide is produced from L-arginine by nitric oxide synthases: NOS3 in endothelium, NOS2 in activated immune cells, and NOS1 in neurons. In activated macrophages, concurrent production of superoxide and NO can lead to peroxynitrite and other reactive nitrogen species (RNS) [39]. RNS can modify protein tyrosine residues, forming 3-nitrotyrosine (3NT), a stable indicator of nitrosative stress [40]. In our study, teff administration after inflammation induction significantly lowered NOx, with TEFF100 and TEFF50 outperforming diclofenac and Trolox in this parameter. Prophylactic benefit on NOx was evident mainly with TEFF100. Teff also reduced 3NT in both protocols, with effects comparable to diclofenac and Trolox. To our knowledge, these findings provide early evidence that teff extract may attenuate RNS-related injury during acute inflammation.

Total thiols represent the combined pool of free and protein-bound sulfhydryl groups, including glutathione and cysteine-containing proteins. Because thiols can directly quench reactive species and help maintain redox balance, they are often treated as an antioxidant capacity marker. In our study, SH increased significantly in TEFF50/INFL and TEFF25/INFL groups, which may relate to dietary antioxidant components and micronutrients present in teff [12,41].

Beyond direct scavenging, teff constituents may also enhance endogenous antioxidant responses. For example, teff grain extracts have been reported to activate the Nrf2 pathway in THP-1 cells, increasing expression of genes involved in glutathione synthesis and recycling [42].

Taken together, the antioxidant activity of teff likely reflects both direct chemical scavenging and indirect upregulation of endogenous defense pathways, which may translate into reduced oxidative injury in conditions linked to redox imbalance [4].

Inflammation is essential for host defense and tissue repair, yet persistent inflammation contributes to multiple chronic diseases. Diet is one modifiable factor influencing inflammatory tone, and whole grains with high fiber and bioactive phytochemicals are increasingly studied for their anti-inflammatory potential. Teff may contribute through fiber, resistant starch, minerals, and antioxidant compounds [4].

Dietary fiber can support beneficial gut microbiota and increase short-chain fatty acid production, which may help regulate immune responses. Teff also contains resistant starch, which has been associated with improved glycemic control and lower inflammatory markers. Its mineral content (especially magnesium) may additionally modulate inflammatory pathways; magnesium deficiency has been linked to higher C-reactive protein levels [4].

Because teff generally has a low glycemic index, it may help avoid rapid postprandial glucose excursions that can activate inflammatory signaling [13].

NF- κ B is a central transcription factor in inflammatory regulation. In resting cells, it is retained in the cytoplasm by I κ B proteins; upon stimulation (e.g., cytokines, oxidative stress, PAMPs/DAMPs), I κ B is degraded and NF- κ B translocates to the nucleus to induce

pro-inflammatory genes. NF- κ B activation can also enhance iNOS expression and NO production, linking inflammation and nitrosative stress [43]. Direct studies on teff and NF- κ B signaling are limited, but teff contains flavonoids (luteolin, apigenin, quercetin) and other polyphenols that are known in other systems to suppress NF- κ B-regulated mediators such as TNF- α and IL-1 β . By lowering oxidative stress, these compounds may reduce upstream triggers that activate inflammatory cascades [4,24].

Additional anti-inflammatory mechanisms may involve microbiota-derived metabolites (e.g., butyrate from fiber fermentation) and mineral status. Magnesium can inhibit NF- κ B signaling, whereas zinc deficiency may enhance it [44].

In our experiments, teff reduced NF- κ B-p65 in both therapeutic and prophylactic protocols, with stronger effects in the prophylactic setting. TEFF100 and TEFF50 showed greater inhibition than diclofenac or Trolox in this preventive model. Nonetheless, human data linking teff intake with NF- κ B activity are limited, and clinical studies are needed to validate these observations.

The NLRP3 inflammasome is an intracellular protein complex that senses diverse danger signals and promotes caspase-1 activation, leading to processing of IL-1 β and IL-18 and, in some contexts, pyroptotic cell death. Dysregulated or persistent NLRP3 activation contributes to inflammatory pathology across multiple diseases [45,46].

IL-18 differs from IL-1 β in that its precursor may be present in several healthy tissues, including blood monocytes and gastrointestinal epithelium. After caspase-1 activation, mature IL-18 is released, while a substantial proportion of the precursor can remain intracellular [47].

Excessive NLRP3 signaling has been implicated in disorders such as gout, atherosclerosis, type 2 diabetes, neurodegenerative disease, and autoimmune conditions. Dietary antioxidants and anti-inflammatory phytochemicals have been proposed as modulators of inflammasome activity [48].

Although direct evidence linking teff intake to NLRP3 modulation is limited, teff's antioxidant profile could reduce oxidative stress, a known trigger for inflammasome activation. In our model, we assessed indirect NLRP3-related markers by measuring IL-1 β , IL-18, and caspase-1. White teff extract reduced IL-1 β in both protocols (with smaller effects than diclofenac and Trolox), inhibited IL-18 (with stronger preventive than therapeutic effects), and lowered caspase-1 (less than diclofenac). Overall, the biomarker pattern is consistent with partial attenuation of NLRP3-related inflammatory activation by white teff extract.

IL-10 is a pleiotropic immunoregulatory cytokine that helps constrain inflammation, in part by suppressing NF- κ B target gene expression and downregulating pro-inflammatory mediators [49,50]. Evidence directly assessing teff's influence on IL-10 is limited. In our acute turpentine model, IL-10 changes were not significant, and teff did not modify IL-10 levels in either protocol.

Together, our results indicate that phenolic acids and flavonoids in white teff extract are associated with measurable anti-inflammatory activity, reflected by reductions in NF- κ B-p65, IL-1 β , IL-18, and caspase-1 without a detectable increase in IL-10. This aligns with prior discussions suggesting teff polyphenols may support immune and inflammatory balance [12].

In summary, white teff extract showed concurrent antioxidant and anti-inflammatory effects in an acute inflammation model. By reducing oxidative stress and dampening pro-inflammatory signaling, teff may offer supportive value for conditions in which chronic inflammation and redox imbalance coexist. These findings reinforce teff's potential role as a functional food component [4,16] and support further investigation of its contribution to cardiometabolic risk reduction when consumed as part of a balanced diet [44].

4. Materials and Methods

4.1. Chemicals

Folin–Ciocâlteu reagent, quercetin, gallic acid, standard chlorogenic acid (>98% HPLC), luteolin, and apigenin (>99% HPLC) were purchased from Sigma-Aldrich (St. Louis, MO, USA); Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Alfa-Aesar (Karlsruhe, Germany); acetonitrile of HPLC purity was obtained from Merck (Darmstadt, Germany); ultrapure water for the HPLC analysis was purified using the Direct-Q UV system from Millipore (Billerica, MA, USA); methanol, ethanol, ferrous ammonium sulfate, vanadium chloride (III) (VCl₃), sulfanilamide (SULF), N-(1-Naphthyl)ethylenediaminedihydrochloric acid (NEDD), sodium nitroprusside (SNP), hydrochloric acid, hydrogen peroxide (H₂O₂), sulphanilic acid, ortho dianisidine dihydrochloric acid (3,3'-dimethoxybenzidine), o-phthalaldehyde, thiobarbituric acid, and xylenol orange [ocresosulfonphthalein-3,3-bis (sodium methyliminodiacetate)] were obtained from Merck (Darmstadt, Germany); the rat ELISA kits were obtained from Elabscience Bionovation Inc. (Houston, Texas) and MyBiosource (San Diego, CA, USA).

4.2. Plant Material Collection and Extract Preparation

Eragrostis tef flour (AGRO DELIVERY SRL, Chitila, Romania) was extracted with 70% (v/v) ethanol using a modified Squibb cold-repercolation procedure performed at room temperature. The resulting extract was prepared at a 1:1 ratio (1 g flour per 1 mL solvent; w:v) [51].

4.3. Phytochemical Analysis

4.3.1. Total Polyphenol Content

Total polyphenol content (TPC) was determined by a modified Folin–Ciocâlteu assay. Briefly, 2 mL of teff ethanolic extract was diluted 25 times. Then, 1 mL Folin–Ciocâlteu reagent and 10 mL distilled water were added, and the mixture was brought to 25 mL with 290 g/L sodium carbonate solution. After 30 min incubation in the dark, absorbance was recorded at 760 nm. The total phenolic content (TPC) was determined using a gallic acid calibration curve (10–50–100–150–200–250–300–350–400–450–500 µg/mL) ($R^2 = 0.999$). Results were expressed as mg gallic acid equivalents per g of dry-weight plant flour (mg GAE/g d.w.) [51].

4.3.2. Total Flavonoid Content

Total flavonoid content (TFC) was assessed as previously reported. To 1 mL of teff ethanol extract, 0.3 mL of 5% NaNO₂ was added, followed by 0.3 mL of 10% AlCl₃. Next, 2 mL of 1 M NaOH was added and the volume was adjusted to 10 mL with distilled water. After 15 min incubation, absorbance was measured at 510 nm. TFC was quantified via a quercetin standard curve (1–5–10–15–20–25–30–35–40–45–50 µg/mL) ($R^2 = 0.999$). Values were expressed as mg quercetin equivalents per 100 g of dry-weight plant material (mg QE/100 g d.w.) [51].

4.3.3. High-Performance Liquid Chromatography Coupled with Electrospray Ionization Mass Spectrometry (HPLC-ESI MS) Analysis

The teff ethanolic extract was profiled by HPLC-DAD coupled to single-quadrupole MS (Agilent 1200 HPLC with DAD; Agilent 6110 MS, Santa Clara, CA, USA). Separation was performed on an Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm) at room temperature. Mobile phase A consisted of 0.1% acetic acid in water with acetonitrile (99:1, v/v), and mobile phase B consisted of 0.1% acetic acid in acetonitrile (v/v). The gradient was as follows: 95% A (0–2 min), 95–60% A (2–18 min), 60–10% A (18–20 min), 10% A (20–24 min),

return to 95% A in 1 min and hold for 5 min [15]. The flow rate was 0.5 mL/min. DAD chromatograms were recorded at 280 nm (phenolic acids) and 340 nm (flavonoids). MS detection used ESI in positive mode (350 °C, 3000 V capillary voltage, nitrogen 8 L/min) with scanning from 100 to 1000 m/z . Compounds were tentatively identified using UV–Vis spectra, retention times, co-chromatography with available standards (Sigma-Aldrich, St. Louis, MO, USA), mass spectra, and literature data. Prior to injection, the lyophilized extract was dissolved in MeOH. Quantification relied on external calibration curves constructed from five concentrations of standards in methanol: chlorogenic acid for phenolic acids ($R^2 = 0.9937$; LOD 0.41 $\mu\text{g/mL}$; LOQ 1.64 $\mu\text{g/mL}$), luteolin for flavones ($R^2 = 0.9972$; LOD 0.26 $\mu\text{g/mL}$; LOQ 0.95 $\mu\text{g/mL}$), and rutin for flavonols ($R^2 = 0.9981$; LOD 0.21 $\mu\text{g/mL}$; LOQ 0.84 $\mu\text{g/mL}$) [51].

4.4. In Vitro Antioxidant Activity Analysis

4.4.1. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Capacity

DPPH radical scavenging activity was assessed using a standard protocol. In brief, 3 mL of teff extract were mixed with 1 mL of 0.1 mM DPPH solution in methanol and incubated for 30 min in the dark at room temperature. Absorbance was measured at 517 nm. Antioxidant activity was calculated as $\text{AA\%} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$. A Trolox standard curve (5–10–20–30–40–50–60–70–80–90–100 $\mu\text{g/mL}$) ($R^2 = 0.999$) was used, and IC₅₀ values were expressed as μg Trolox equivalents per g of dry-weight flour extract ($\mu\text{g TE/g d.w.}$) [51].

4.4.2. Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing antioxidant power (FRAP) was measured as described previously [52]. A 100 μL aliquot of teff ethanolic extract was mixed with 3.4 mL FRAP reagent. After 30 min, absorbance was read at 593 nm, and results were reported as μg Trolox equivalents per g of dry-weight plant flour extract ($\mu\text{g TE/g d.w.}$) using a Trolox standard curve (25–50–75–100–125–150–175–200 mg/mL) ($R^2 = 0.999$).

4.4.3. Hydrogen Peroxide (H_2O_2) Scavenging Activity

Hydrogen peroxide (H_2O_2) scavenging activity was evaluated following a published method. Teff extract was added to an H_2O_2 solution and, after 10 min, absorbance was measured at 230 nm against phosphate buffer. Scavenging percentage was calculated as $(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100$. Results were reported as IC₅₀ in mg Trolox equivalents per g of dry-weight plant flour extract (mg TE/g d.w.) using a Trolox standard curve (10–20–30–40–50–60–70–80–90–100 $\mu\text{g/mL}$) ($R^2 = 0.999$) [51].

4.4.4. Nitric Oxide (NO) Radical Scavenging Assay

Nitric oxide (NO) scavenging was determined using sodium nitroprusside (SNP) as the NO donor. Teff extract (0.5 mL) was combined with SNP solution (2 mL SNP + 0.5 mL PBS, pH 7.4) and incubated for 2.5 h at 25 °C. Then, 0.5 mL of the reaction mixture was mixed with 1 mL sulphanilic acid; after 5 min, 1 mL N-(1-naphthyl)ethylenediamine dihydrochloride was added. Samples were vortexed and incubated in the dark for 30 min, and absorbance was measured at 546 nm. Inhibition was calculated as $(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}} \times 100$. Results were expressed as IC₅₀ in μg quercetin equivalents per g of dry-weight plant flour ($\mu\text{g QE/mL d.w.}$) [51].

All *in vitro* assays were performed in triplicate. Spectrophotometric measurements were obtained using a UV–Vis spectrophotometer (Jasco V-350, Jasco International Co., Ltd., Tokyo, Japan).

4.5. In Vivo Experimental Design

4.5.1. Experimental Protocol

Adult male Wistar rats (200–250 g) were obtained from the Establishment for Breeding and Use of Laboratory Animals, “Iuliu Hațieganu” University of Medicine and Pharmacy (Cluj-Napoca, Romania). Animals were housed under standard conditions (25 ± 1 °C; $55 \pm 5\%$ relative humidity; 12 h light/dark cycle) with ad libitum access to standard chow and water. Rats were randomized into 10 groups ($n = 9$). The extract was tested at three doses of dry teff flour extract per mL: 100 mg/mL (abbreviated as TEFF100), 50 mg/mL (abbreviated as TEFF50), and 25 mg/mL (abbreviated as TEFF25). All treatments were administered by oral gavage in a volume of 1 mL/rat/day and prepared fresh each day. The SHAM group served as a healthy control without treatment. For the treatment protocol in six groups, acute inflammation was induced on day 1 by intramuscular turpentine oil (6 mL/kg b.w.). For the subsequent 10 days, treatments were administered orally (gavage) as follows: INFL group received tap water (1 mL/rat/day); DICLO group received diclofenac (10 mg/kg b.w./day); TX group received Trolox (50 mg/kg b.w./day); INFL/TEFF100 (500 mg/kg/day), INFL/TEFF50 (250 mg/kg/day), and INFL/TEFF25 (125 mg/kg/day) [7]. For the prophylactic protocol (TEFF100/INFL, TEFF50/INFL, TEFF25/INFL), the corresponding teff flour extract doses were given for 10 days, and inflammation was induced on day 11. On day 12, rats were anesthetized with ketamine (60 mg/kg b.w.) and xylazine (15 mg/kg b.w.) [52]; blood was collected by retro-orbital puncture, serum was separated, and samples were stored at -80 °C until analysis.

The project was approved by the Veterinary Sanitary Direction and Food Safety Cluj-Napoca (No. 372/04.07.2023). All the procedures comply with Directive 2010/63/EU and Romanian national law 43/2014 for the protection of animals used for scientific purposes.

4.5.2. Oxidative Stress Biomarkers Assessment

Total Oxidative Status (TOS)

Total oxidative status (TOS) was quantified based on the oxidation of ferrous ions to ferric ions in an acidic environment in the presence of reactive oxygen species. Absorbance was measured at 560 nm and results were expressed as $\mu\text{mol H}_2\text{O}_2$ equivalents/L ($\mu\text{M H}_2\text{O}_2$ equiv/L) [52].

Total Antioxidant Capacity (TAC)

Total antioxidant capacity (TAC) was assessed by measuring suppression of hydroxyl radical generation in a Fenton-type reaction by antioxidants present in serum. Absorbance was recorded at 444 nm and results were reported as mmol Trolox equivalents/L (mM TE/L) [53].

Oxidative Stress Index (OSI)

Oxidative stress index (OSI) was calculated as the ratio between oxidants and antioxidants: $\text{OSI (arbitrary units)} = \text{TOS (mM H}_2\text{O}_2 \text{ equiv/L)} / \text{TAC (mM Trolox equiv/L)}$. This index provides an integrated estimate of oxidative stress burden [54].

Advanced Oxidation Protein Products (AOPP)

Advanced oxidation protein products (AOPP) were measured as a protein oxidation marker using a spectrophotometric assay [55]. Samples and chloramine-T (blank) were diluted to 10% in PBS, and then potassium iodide and glacial acetic acid were added. Absorbance was read at 340 nm and concentrations were expressed as μM chloramine-T equivalents/L.

Malondialdehyde (MDA)

Malondialdehyde (MDA), as an index of lipid peroxidation, was determined using a thiobarbituric acid-based method [56]. In brief, 0.1 mL serum was mixed with 0.1 mL 40% trichloroacetic acid and 0.2 mL 0.67% thiobarbituric acid, heated for 30 min in a boiling water bath, cooled in ice, and centrifuged for 5 min at 3.461 g. Absorbance was measured at 532 nm and MDA concentration was expressed as nM/mL serum.

Nitric Oxide Synthesis (NO)

Nitric oxide production was estimated by determining total nitrites and nitrates (NO_x) via the Griess reaction. Serum proteins were removed by extraction with methanol/diethyl ether (3:1, *v/v*), nitrates were reduced to nitrites with vanadium (III) chloride, and absorbance was measured at 540 nm. Results were expressed as nitrite $\mu\text{M/L}$ [56].

3-Nitrotyrosine (3NT)

3-Nitrotyrosine (3NT), a marker of peroxynitrite-mediated oxidative damage [56], was quantified using an ELISA kit (E-EL-0040) according to the manufacturer's protocol. Results were expressed as ng/mL.

Total Thiols (SH)

Total thiols (SH) were determined with Ellman's reagent [57]. Absorbance was read at 412 nm, and concentrations were expressed as mM glutathione equivalents per mL (mM GSH/mL).

4.5.3. Inflammatory Biomarkers Assessment

Systemic inflammatory response was evaluated by ELISA quantification of NF κ B-p65 (E-EL-RO674), IL-1 β (E-EL-0012), IL-18 (E-EL-R0567), caspase-1 (E-EL-R0371), and IL-10 (E-EL-R0016). Assays were performed according to manufacturers' instructions and results were reported as pg/mL.

Spectrophotometric measurements were performed on a UV-Vis spectrophotometer (Jasco V-350, Jasco International Co., Ltd., Tokyo, Japan). ELISA procedures used a Biotek microplate 50 TS washer and an 800 TS microplate reader (Agilent Technologies Inc., Santa Clara, CA, USA).

4.6. Statistical Analysis

Data are presented as mean $\pm$ standard deviation (three independent determinations) when normally distributed. Group comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. Pearson correlation and principal component analysis (PCA) were applied to explore relationships between oxidative stress and inflammatory markers. Statistical significance was set at $p < 0.05$. Analyses were conducted in SPSS Statistics v26.0 (SPSS, Chicago, IL, USA).

5. Conclusions

In conclusion, the white teff flour ethanolic extract shows promise as a nutraceutical candidate, with the potential to support inflammatory conditions through simultaneous reductions in inflammatory biomarkers and nitro-oxidative stress.

As research on teff expands, additional health-relevant mechanisms may be clarified, particularly in relation to oxidative stress pathways and chronic disease risk. At present, teff remains an attractive gluten-free ancient grain that combines nutritional density with bioactive constituents, supporting its emerging role in functional food development.

Further well-designed human studies are needed to confirm these effects, clarify the bioavailability and metabolic fate of teff antioxidants, and define how teff-based dietary strategies can be integrated into the management of inflammatory diseases.

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