



Tridax Procumbens as a potential immunomodulator: A comprehensive review of phytoconstituents and pharmacological evidences

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Abstract

This critical review passes the direct and indirect substantial for the immunomodulatory activity of *T. procumbens* using classic humoral and cell-mediated assays, bioactivity-directed fractionation, isolated constituents, inflammatory signal transduction (cyclooxygenase-2, inducible nitric oxide synthase, nuclear factor- κ B), oxidative stress, toxicological profiling, and contemporary preparations.

In vivo immunostimulation studies are rodents are an ethanol-insoluble fraction of aqueous extracts provided significant increases in peritoneal phagocytosis, total leucocyte counts, primary haemagglutination antibody titre, and plaque-forming splenic cells, with enhanced delayed-type hypersensitivity (DTH) responses. Bioactivity-directed fractionation showcase the immunological activity in medium-to-high polarity ethyl acetate and n-butanol fractions. In contrast, *ex vivo* assays using human peripheral blood mononuclear lymphocytes from systemic lupus erythematosus patients showed disease-state dependent immunosuppression with IgG secretion inhibited in healthy volunteers and quiescent SLE at concentrations above 62.5 μ g/mL, but increased in active SLE patients. Characterized flavonoids, coumarins, polyacetylenes, and phenolic acids modulate inflammatory cyclooxygenases, cytokine production, and macrophage redox status.

Keywords: *Tridax procumbens*, immunomodulation, immunostimulation, immunosuppression, humoral immunity, cell-mediated immunity, delayed-type hypersensitivity, flavonoids, NF- κ B signaling, cyclooxygenase-2, phytopharmaceuticals

Introduction

Immune modulation as refers to the change in the immune function they may enhance, reduce, or restore an organism's immunologic response to a particular stimulus. There is a growing need in studying medicinal plants' role in this process since they may have multiple effects on different pathways rather than interacting with one specific receptor. One such herb with reported immunomodulatory effects is *T. procumbens*, as there are various reports of its anti-inflammatory, antioxidant, antimicrobial, healing promotion, and immunological effects. In fact, more than 138 constituents were described in a 2022 study, including flavonoids and terpenoids. However, the possible variety of biological activities may complicate the treatment course, since different parts of the plant or extracted with different solvents may have varying compounds.^[30]

The most convincing evidence of the immunostimulatory effects of the plant comes from animal studies. Tiwari *et al.*, when injected with an ethanol-insoluble aqueous fraction of the extract, found that the test subjects had increased phagocytosis, leukocyte counts, antibody-secreting cell frequency, hemagglutination titers, and delayed-type hypersensitivity. Moreover, the researchers report that ethyl-acetate and n-butanol fractions may have similar effects.^[1, 2, 3]

However, one study noted that the herb may differently affect IgG secretion for healthy and SLE lymphocytes – while higher concentrations inhibited the protein production for normal and stable SLE cells, the active SLE cells displayed increased secretion.^[4] Thus, for cellular immunity, it may not be appropriate to call this substance immunostimulatory, but rather “immunomodulatory”.

Objectives, Literature Strategy and Evidence Grading

The review was structured around five objectives: identify direct immune endpoints; compare extracts and fractions; associate biological activity with phytochemical classes; evaluate inflammatory and antioxidant mechanisms; and define a development pathway for standardized formulations.

Primary experimental reports were prioritized for biological conclusions. Reviews were used to chart the literature and identify supporting studies. Evidence was compared using plant part, solvent, fraction, route, dose, model and endpoint categories. Direct immune outcomes were weighted more heavily than indirect antioxidant or antimicrobial observations.

Evidence was categorized conceptually: direct when an immune endpoint was measured; mechanistic when pathway or mediator data were provided; supportive when antioxidant, antimicrobial or phytochemical data suggested plausibility; and translational when formulation, pharmacokinetics or human data were available.

Conceptual Assessment Structure

Tier 1: Primary Immune Results: *In vivo* or *in vitro* research directly evaluating functional leukocyte activity (e.g., carbon elimination rates, delayed-type allergy responses, plaque-creating cell counts, quantitative immunoglobulin ELISA).

Tier 2: Mechanistic / Pathway Validations: Tests assessing transcriptional regulators, suppression of inflammatory mediators (iNOS, COX-2, PGE2) and profiles of cytokines.

Tier 3: Supportive Observations: *In vitro* biochemical antioxidant assessments (DPPH, ABTS) and antimicrobial tests aimed at pathogens.

Tier 4: Translational & Safety Formulations: Pharmacokinetic, toxicological, and innovative carrier

transport information (e.g., chitosan gels, green metal nanoparticles).

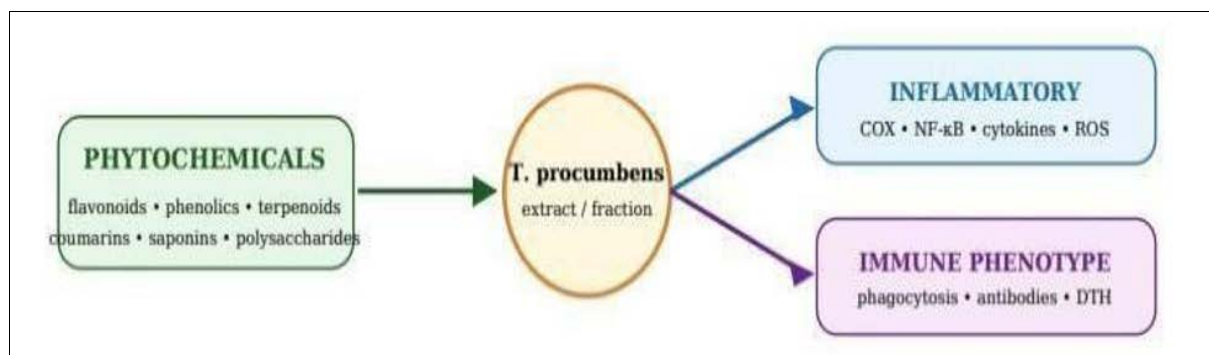


Fig 1: Original evidence map connecting phytochemical composition with inflammatory and immune phenotypes.

Botanical and Pharmacognostic Context

Taxonomy and morphology: *T. procumbens* belongs to Asteraceae and is a procumbent herb Found widely in tropical and subtropical environments. Leaves, flowers, aerial parts and Whole plant preparations have all been studied. Authentication is essential because Environmental conditions and post-harvest handling can alter phytochemical composition, Plant-part effects. Leaves are common in topical and antioxidant studies, aerial parts are Frequent in standardized anti-inflammatory investigations, and whole-plant material has been used in metabolic studies. These differences should be treated as different interventions rather Than interchangeable sources of the same drug.

Extraction variables: Aqueous extraction favours polar compounds, whereas methanol, ethanol And ethyl acetate recover broader or more lipophilic chemical fractions. Fractionation can enrich active constituents but can also destroy synergistic combinations. Every pharmacological Study should therefore report solvent composition, plant-to-solvent ratio, extraction time and Temperature, yield and storage. Quality control. A publication-ready botanical preparation should have a voucher specimen, Identity test, chromatographic fingerprint, marker-compound range, moisture and microbial Specifications, and contaminant testing appropriate to the route of administration.

Immunological Rationale and Experimental Endpoints:

Immune Basis and Outcomes of Interest Innate immunity reflects rapid responses and clearance by cells including Neutrophils and macrophages, whereas adaptive immunity includes antigen specific B- And T-cell responses. These are interdependent systems, thus an immunomodulator ideally should be assessed using multiple endpoints.

Leukocyte counts, and phagocyte indexes provide general indices of innate activity. Antibody secreting Cells and Possible spelling mistake found. Titles reflect humoral outcomes, delayed-type Hypersensitivity, provides a functional assessment of cell mediated activity. No single assay is Sufficient to demonstrate therapeutic benefit.

Inflammation is intimately involved with immunity, but the two terms are not synonymous. An extract with COX-2 or NFL inhibitory activity potentially could reduce inflammatory tone without global suppression of host defense mechanisms. On the other hand, excessive Immunosuppression might cause inadequate control of

pathogens. This distinction is critical in the interpretation of results of studies using plant extracts.

Subsequent investigations should include evaluation of immune-cell function plus cytokine Profiles, biochemical assays of relevant pathways, determination of cell viability and appropriate positive/ Negative controls. Experimental models should involve reporting of sex, strain, age, dose, Route, randomization and blinding procedures, and adverse events.

Direct Immunostimulatory Evidence

Tiwari and colleagues demonstrated one of the most compelling direct experimental findings that an ethanol-insoluble fraction of a water-based *T. procumbens* extract was administered intraperitoneally to test animals at doses of 0.25 and 0.5 g/kg which they found to be associated with an increase in the phagocytic index, white blood cell numbers and splenic antibody-producing cells, and increased haemagglutination titers and delayed-type hypersensitivity.^[1]

The strength of this study lies in the convergence of end-point results for innate and adaptive immune responses which renders it more convincing than a single measure of haematological activity. It functionally explore functional responses to both antigen and anaphylactic challenge. Nevertheless, the route of administration and dosing limits its translation to human scenarios. The fraction was not characterized by a contemporary marker set, and the active chemical constituents were not determined completely. Therefore, the clinical trial shows biological efficacy but is far from a recommendation for a clinical dosage or a standardized formulation.

It subsequently described immunomodulatory actions of an ethanolic extract in rats challenged by *Pseudomonas aeruginosa* which involved alterations in humoral immunity and the process of engulfing particles.^[5] Due to the inclusion of infection as part of the model, its direct antimicrobial effects and immune modulation cannot be fully distinguished.

Fractionation and Bioactivity-Guided Evidence

Fractionation helps us move past crude-extract screening. A methanolic extract was split into chloroform and ethyl-acetate, n-butanol and aqueous fractions. The ethyl-acetate and n-butanol fractions showed immunomodulation activity and we then pursued the chosen compounds with bioactivity-guided isolation.^[2, 3]

Fractionation is important because it builds a bridge between chemistry and pharmacology. When activity remains with a fraction through repeated purification the chance of a real chemical-biological relationship rises. Fractionation can also pull out helpful partners so if activity disappears after purification it does not prove that the isolated compound was useless, in the original mixture. A modern bioactivity-guided program should mix chromatography with tests at every step. LC-MS or LC-MS/MS can track candidate markers while immune

assays can tell us if potency comes from one compound, a cluster of compounds or a broader chemical class. Chemical standardization must be created with biological standardization. A marker panel is most useful when its amount predicts batch potency. If marker amount changes but biological activity stays the same the marker is not enough; if biological activity changes while the marker stays constant we must investigate components or process variables.

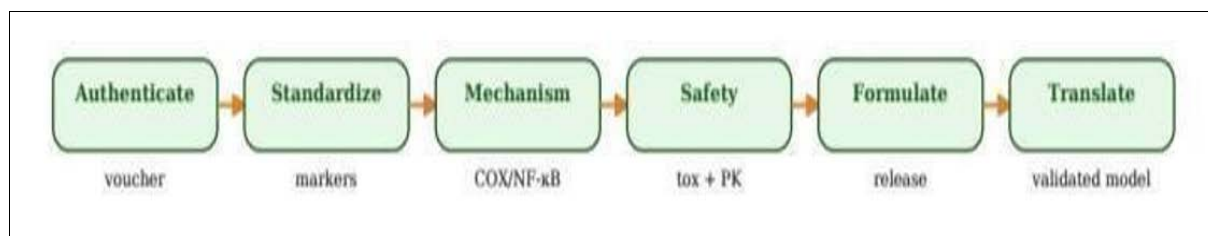


Fig 2: Original workflow from authentication to formulation and translation.

Humoral and Cellular Immunity

The increase in antibody-secreting cells and hemagglutination titers shows that certain fractions of *T. procumbens* may boost immune responses when tested in experimental models. This finding is supported by delayed-type hypersensitivity, which points to cell-mediated immune activity.^[1] The combination of these effects is more meaningful than a rise in total leukocytes because it suggests actual changes in immune function when measuring antibodies it's important to know whether the increase comes from more cells producing antibodies or from changes in cell survival or activity. Delayed-type hypersensitivity results should be interpreted carefully with proper controls and if possible with cytokine profiling or tissue analysis to confirm the immune response. Neutrophil adhesion and phagocytosis are screening tools but they can be influenced by the color of the extract, precipitation, osmolarity or toxicity. Future studies with cells should include vehicle controls, tests for cell viability and multiple biological replicates to ensure reliable results. In infection models it's crucial to immune effects from direct antimicrobial actions. A treatment that lowers load might cause immune markers to return to normal which could be mistaken for immune enhancement. On the hand better clearance of the pathogen may simply reflect a stronger immune response. For this reason it's best to measure pathogen levels immune-cell activity and inflammatory signals, at the time. This way the true nature of the response can be better understood.

Humoral Immunity: Antibody Titers and Splenocyte Proliferation

Administration of *T. procumbens* crude extracts and purified flavonoid/saponin fractions enhances humoral immune responsiveness against antigen challenge^[1, 2]. In rodents immunized with sheep red blood cells (SRBC), animals pre-treated with *T. procumbens* extracts (100–500 mg/kg p.o.) Exhibit significantly higher primary and secondary hemagglutinating antibody (HA) titers compared to control animals (Tiwari *et al.*, 2004)^[1]. This augmentation is driven by increased Blymphocyte proliferation in the splenic germinal centers and enhanced

Differentiation into immunoglobulin-secreting plasma cells, mediated in part by balanced helper T-cell cytokine signaling (IL-4 and IFN- γ release)^[1, 2].

Cell-Mediated Immunity: Delayed-Type Hypersensitivity (DTH)

Cell-mediated immune responses are assessed via the Delayed-Type Hypersensitivity (DTH) reaction using SRBC or 2,4-dinitrofluorobenzene (DNFB) footpad challenge^[1,2]: T-cell primed animals receiving *T. procumbens* show an increased footpad swelling thickness at 24 and 48 hours post-antigen challenge^[1].

Histological examination of DTH reaction sites reveals marked recruitment of CD4+and CD8+ T lymphocytes and monocytes, Confirming that the extract facilitates T-cell homing and sensitized effector recall^[1, 2].

In models of cyclophosphamide-induced immunosuppression, *T. procumbens* prevents severe depletion of white blood cells (leukopenia), preserves CD4/CD8 ratios, and maintains bone marrow cellularity^[1].

Innate Immunity: Phagocytic Stimulation and Macrophage Priming

The most consistently validated innate immunomodulatory feature of *T. procumbens* is its capacity to amplify phagocytic clearance. In Polymorph nuclear (PMN) neutrophil function assays using human peripheral blood, aqueous and ethanol-insoluble fractions significantly augment the phagocytic index against *Candida albicans* and *Staphylococcus aureus*^[2].

Intracellular Microbial Killing: *T. procumbens* extracts enhance lysosomal enzyme degranulation and promote calibrated Superoxide production via NADPH oxidase without triggering cytotoxic hyper-inflammation^[2].

Carbon Clearance & Reticuloendothelial Stimulation: In *in vivo* rodent models, oral administration of *T. procumbens* extracts significantly increases the phagocytic index (\$K\$) in the carbon clearance test, indicating stimulated reticuloendothelial system (RES) activity in the spleen and liver^[1, 2].

Dual Polarization: While promoting baseline antimicrobial clearance in resting macrophages, the extract suppresses

excessive Pro-inflammatory polarization in LPS-activated macrophages, reducing inducible nitric oxide synthase (iNOS) transcription and Attenuating elevated levels of IL-1 β , IL-6, and TNF- α [36].

Autoimmune Context and Immunosuppressive Findings

Purba *et al.* evaluated an ethyl-acetate extract of *T. procumbens* in cultured lymphocytes from a healthy volunteer and patients with stable or active systemic lupus erythematosus. IgG secretion was measured by ELISA across a concentration range. [4]

In healthy and stable-SLE lymphocytes, concentrations above 62.5 μ g/mL reduced IgG secretion, whereas the active-SLE preparation showed an opposite response, with IgG increasing across the tested concentrations. [4] This disease-state dependence is a major reason that the plant should be described as an immunomodulator rather than an immune Stimulant.

The observation is compatible with a context-dependent signaling model. Autoimmune lymphocytes may differ in activation state, cytokine environment, metabolism and pathway sensitivity. A compound mixture that suppresses one signaling route in a stable state may behave differently in an actively inflamed state.

The supplied manuscript connects the SLE finding with COX biology because activated human B lymphocytes express COX-2 and COX inhibition can attenuate antibody production. [6] This is a plausible mechanistic link, not yet a direct demonstration that COX inhibition explains the SLE response.

COX 2, NF κ B and Inflammatory Signaling

Jachak *et al.* Examined methanol and ethanol extracts from the aerial parts of the plant. They tested these extracts in a rat paw edema model well as in COX inhibition assays. The ethyl acetate extract proved to be the strongest; it produced the inhibition of both COX 1 and COX 2 at the concentration tested. From this extract the researchers isolated three compounds: centaurein, centaureidin and bergenin. The extracts also displayed activity as measured by DPPH and ABTS radical scavenging tests. [6]

Berlin Grace *et al.* Found that treating albino mice with a leaf extract lowered TNF α and COX 2 gene expression, at sites of induced inflammation. [19] These results give tissue level support to the pathway hypothesis. However they do not prove that COX 2 modulation alone explains the immunological changes.

Recent chemical studies give mechanistic insight. Zheng *et al.* Isolated 22 coumarins, seven of which were compounds and tested them in LPS stimulated RAW 264.7 macrophages. Several of these compounds reduced nitric oxide production. In particular compound 6 lowered the expression of iNOS, COX 2, IL 1 β and IL 6 and it also blocked the translocation of NF κ B. [8] Taken together these studies point to a plausible network that connects phytochemicals with inflammatory mediators. The proper conclusion is that COX and NF κ B signaling represent a mechanistic target. It does not mean that every preparation of *T. Procumbens* works COX 2, NF κ B and Inflammatory Signaling Jachak *et al.* Examined methanol and ethanol extracts from the aerial parts of the plant.

Phytochemical Basis of Immunomodulation

Chemical investigations describe flavonoids, phenolics, terpenoids, saponins, sterols, tannins, polysaccharides, coumarins, polyacetylenes and other metabolites. The 2022 comprehensive review summarized more than 138 reported constituents, illustrating the breadth of the chemical system. [30]

Flavonoids and phenolics are repeatedly implicated. Cui *et al.* isolated six flavonoid-related compounds and reported significant antioxidant/free-radical-scavenging activity for several isolates. [20] Saxena *et al.* found antioxidant activity in purified ethyl-acetate and n-butanol fractions and related activity to phenolic and flavonoid content. [7]

Terpenoids and sterols broaden the chemical profile toward less polar fractions, helping explain why ethyl-acetate or other medium-polarity fractions can retain activity. Polysaccharides provide a second chemical scale: a sulphated polysaccharide from *T. procumbens* showed antibacterial and antiherpetic activity. [10]

Recent work demonstrates that the chemical space is still expanding. Zheng *et al.* isolated 22 coumarins in 2026, while Tan *et al.* isolated 26 polyacetylenes, including 17 previously undescribed compounds. [8, 24] These discoveries create new lead opportunities but also make standardization more important.

Antioxidant and Oxidative Stress Evidences

Oxidative stress can shape signals change membrane lipids and alter how immune cells work. Antioxidant activity is therefore important for controlling the system but the tests that look for free-radical scavenging cannot be taken as proof of real immune power.

Jachak and colleagues showed that extracts that were standardized could stop DPPH and ABTS radicals and they argued that this antioxidant action helped create a picture. [6] Saxena found DPPH activity in fractions while Cui *et al.* discovered flavonoids that act as antioxidants. [7, 20] Offered evidence showing that the extracts could lower uric acid act as antioxidants and fight bacteria. [21] Recent work with a gel that uses *T. Procumbens* and chitosan kept its power when tested with DPPH and hydrogen peroxide. [22]

To make the story stronger future studies should check the amount of oxygen species inside cells measure glutathione levels look at lipid damage and count antioxidant enzymes in immune cells. Then the researchers should link these inside-cell changes to cytokine levels or how the immune cells actually act. Chemical tests such as DPPH and ABTS are useful as clues. Not, as final proof. Chemical radical-scavenging $\rightarrow$ redox effects $\rightarrow$ pathway modulation $\rightarrow$ functional immune response. Each step requires validation.

Antimicrobial and Antileishmanial Evidence

Taddei and Rosas-Romero reported solvent- antibacterial activity in crude extracts with activity against organisms including *Escherichia coli*, *Mycobacterium smegmatis*, *Salmonella* spp., *Bacillus cereus* and *Klebsiella*. [28, 29] The 2020 leaf study also found activity across solvent extracts. [9]

Fokialakis *et al.* Investigated *T. Procumbens* against *Leishmania mexicana* promastigotes and isolated an oxylipin with activity. [11] Gamboa-Leon *et al.* Later tested *T. Procumbens* with *Allium sativum* in mice. Found that the combination controlled lesion development more effectively than the individual treatments. The mixture was associated

with a IgG2a/IgG1 ratio suggesting a Th1-oriented immune response.^[12]

These studies illustrate the possibility of action: direct pathogen inhibition plus host-response modulation. However the two mechanisms should be quantified separately. Pathogen load, cytokines antibody subclasses, immune-cell activity and tissue pathology should be measured in parallel.

Naqash and Nazeer found that a sulphated polysaccharide had antiherpetic effects and was nontoxic to Vero cells up to the tested concentration.^[10] Such macromolecular activity broadens the rationale but does not replace standardized immunological testing.

Wound Healing, Safety and Toxicology

Yaduvanshi *et al.* evaluated a topical leaf-juice ointment in mice and found dose dependent effects. A lower concentration produced healing comparable to a VEGF ointment, whereas the higher concentration induced inflammation, edema and reduced vascularity.^[13]

This is an important lesson for immunomodulatory development: the same botanical can have different biological effects at different concentrations. Nandita *et al.* evaluated a *T. procumbens* based chitosan gel and found concentration-dependent anti-inflammatory and antioxidant activity in albumin-denaturation and radical-scavenging assays.^[22] Johnson *et al.* synthesized selenium nanoparticles using stem extract and reported antibacterial, antioxidant and anti-inflammatory activity with low toxicity in their screening model.^[23]

Safety evidence remains less mature than efficacy evidence. The 2022 review noted limited toxicological knowledge. Short-term animal tolerability cannot establish chronic safety, reproductive safety, genotoxicity, herb–drug interaction risk or a therapeutic index. The 2018 hematological study reported low apparent toxicity of an aqueous leaf extract over the tested period, but the finding remains model- and dose-specific.^[27]

For development, acute and repeated-dose toxicity should be accompanied by hematology, clinical chemistry, histopathology and, where relevant, genotoxicity and reproductive studies. Pharmacokinetics should establish systemic exposure to marker compounds and identify whether formulation changes alter exposure.

“No toxicity observed in one experimental model” is not equivalent to “safe for human therapeutic use.” A clinical claim requires a structured safety package.

Formulation and Standardization Strategies

Formulation can improve stability, usability and transportation of materials. Formulation may also change the chemical makeup of plant-based formulations. The candidate platforms outlined in the manuscript extracts, phytosomes, nanoparticles, hydrogels, liposomes and solid-lipid nanoparticles should be seen as options for research and development of recognized medical therapies. Standardized extracts give a basis for evaluating chemicals. Phytosomes can improve the connection between formulation and body membranes; nanoparticles can manage release systems and targeted sites; hydrogels support delivery; liposomes safeguard delicate compounds; and solid-lipid nanoparticles assist in protection and release regulation.

Recent tests suggest that these formulations are successful. A chitosan gel preserved formulation’s anti-inflammatory and radical-scavenging abilities, while selenium nanoparticles synthesized from *T. Procumbens* stem extract exhibited properties, lowered free radicals and lessened inflammation. I find these findings encouraging.^[22, 23] Studies on formulations must encompass information regarding particle size and distribution surface properties when applicable load capacity, release kinetics, stability, purity and strength. A formulation is not considered successful, for creating a small or stable gel; the effectiveness of the botanical must be assessed after processing.

Proposed mechanistic model and research gaps

Working model. The literature shows that there is a multi-layered mechanism of just a single receptor-level explanation. Flavonoids and phenolics may help control balance and inflammation signaling; terpenoids and saponins may change how immune cells behave; certain coumarins and polyacetylenes may act on specific pathways; and complex fractions may keep the interactions among many components.

Gap 1—Chemical definition. I notice that many older studies use extracts that do not contain marker panels. Future experiments should clearly define plant identity, extraction conditions and chromatographic fingerprints before testing activity.

Gap 2— linkage. I see that COX NF-κB, cytokines and antioxidant assays are often reported separately. The next generation should measure these pathways in the standardized preparation and link them to a functional immune phenotype.

Gap 3—Exposure. I realize that in-vitro concentrations cannot be assumed to represent human exposure. Pharmacokinetic studies should quantify marker compounds, metabolites, stability, and absorption and tissue distribution.

Gap 4—Safety. I think that repeated-dose toxicity, genotoxicity, reproductive safety and herb-drug interaction studies remain underdeveloped. These are necessary, before claims.

Gap 5— Reproducibility. I notice that harvest drying, solvent, extraction ratio, temperature and storage should be locked into a manufacturing-style specification.

Gap 6—Translation. I understand that controlled human studies should follow not precede, chemical standardization, safety and pharmacokinetic validation.

Phytochemical composition and synergism

The immunopharmacological versatility of *T. procumbens* stems from its rich diversity of secondary Metabolites, which function through polypharmacological synergies rather than isolated monotherapeutic Pathways.

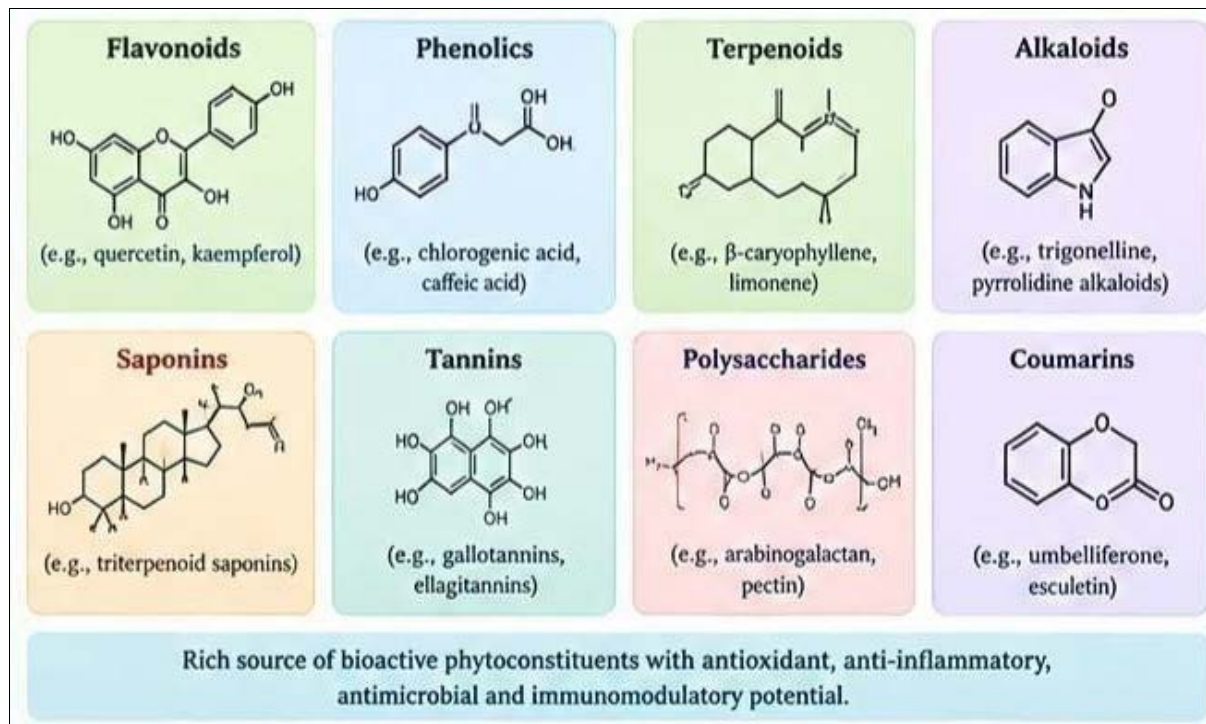
Flavonoids: Quercetin, luteolin, rutin, apigenin, and isorhamnetin represent the predominant flavonoid class. These polyphenols function as potent free-radical scavengers and regulators of pro-inflammatory cytokine Expression.

Terpenoids & Sterols: Significant isolates include tridaxic acid, lupeol, β -amyrin, taraxerol, and Stigmasterol, which exert membrane-stabilizing and anti-edematous actions.

Phenolic Acids: Gallic acid, caffeic acid, chlorogenic acid, and ferulic acid contribute to cellular redox Homeostasis.

Alkaloids: Tridaxonine and tridaxine impart microbicidal and mild immunotropic actions.

Saponins & Glycosides: Oleanolic acid derivatives and quercetin-3-O-glucosides that stimulate macrophage Engulfment kinetics.



Mechanism of Immunomodulation

Experimental investigations confirm that *T. procumbens* acts across five synchronized biological pillars:

1. Innate Immunity Activation

Extracts of *T. procumbens* significantly augment the phagocytic index of peritoneal macrophages in a dose Dependent manner. Additionally, treatment enhances the cytotoxic activity of Natural Killer (NK) cells, stimulates Neutrophil chemotaxis and adhesion, and modulates the respiratory burst to generate controlled microbicidal Reactive oxygen species (ROS) and reactive nitrogen species (RNS) without inducing cytotoxic collateral tissue Damage.

2. Adaptive Immunity Modulation

In humoral immunity models, administration of aqueous and ethanolic fractions enhances hemagglutination Antibody titers (IgG and IgM) against sheep red blood cell (SRBC) challenges. In cellular immunity, the extract Potentiates delayed-type hypersensitivity (DTH) reactions, stimulates lymphocyte blastogenesis, and recalibrates The Th1/Th2 balance by selectively promoting protective Th1 responses (IFN- γ , IL-2) while curbing hyperactive Th2 cascades (IL-4, IL-5).

3. Cytokine Modulation & Signal Transduction

Under hyper inflammatory conditions, *T. procumbens* down regulates the transcription and secretion of pro-

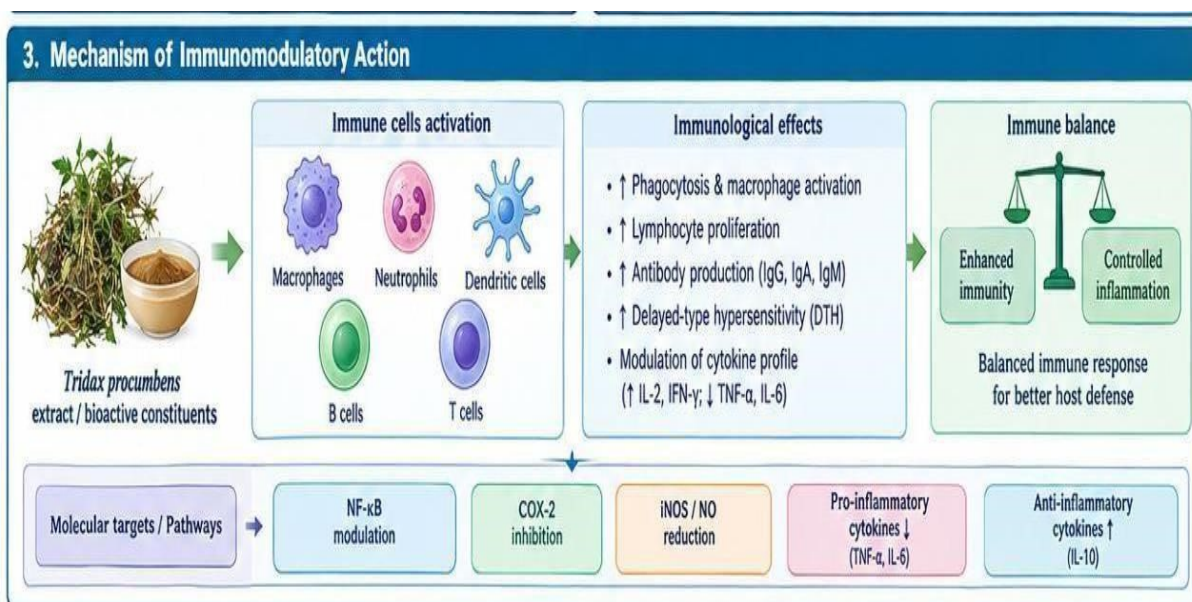
Inflammatory cytokines, specifically Tumor Necrosis Factor-alpha (TNF- α), Interleukin-1 beta (IL-1 β), and Interleukin-6 (IL-6). Mechanistically, this suppression occurs via the inhibition of Nuclear Factor kappa B (NF- κ B) phosphorylation and nuclear translocation of the p65 subunit. Concurrently, it upregulates anti-inflammatory Cytokines, including Interleukin10 (IL-10) and Transforming Growth Factor-beta (TGF- β), and activates the Nrf2/HO-1 antioxidant response pathway.

4. Redox Regulation & Mitochondrial Protection

The phytoconstituents directly scavenge DPPH, ABTS, superoxide radicals, and hydroxyl radicals. In inflamed Tissues, it restores endogenous antioxidant enzyme levels (Superoxide Dismutase, Catalase, and Glutathione Peroxidase) while substantially reducing malondialdehyde (MDA) accumulation, preserving mitochondrial Membrane potential.

5. Tissue Repair & Remodeling

In cutaneous and deep wound models, *T. procumbens* accelerates re-epithelialization by stimulating basic Fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). This facilitates rapid capillary Sprouting, enhanced collagen synthesis (types I and III), and organized cross-linking, significantly increasing Wound contraction rates and tensile strength.



Proposed mechanism map

Flavonoids/phenolics/coumarins and other constituents
↓
Redox regulation + inflammatory signaling modulation
↓
NF-κB/MAPK-related changes and altered mediator expression
↓
Controlled inflammatory response

Pharmacokinetic, Safety & Translational Perspectives

Preclinical acute toxicity assessments establish an oral LD₅₀ > 2000–5000 mg/kg body weight in rodent Models, classifying the extract into Category 5 (unclassified/non-toxic under OECD guidelines). Repeat-dose sub-Chronic

toxicity evaluations (90 days at doses up to 500 mg/kg/day) revealed no observable adverse effect levels across hematological, hepatic, or renal parameters.

To overcome potential bioavailability hurdles of crude phytocomplexes, modern pharmaceutical formulation Strategies including Carbopol-based topical hydrogels, lipid Nano carriers (liposomes, solid lipid nanoparticles), PLGA polymeric nanoparticles are currently being explored. These advanced delivery modalities protect Labile flavonoids from first-pass hepatic degradation, sustain plasma drug concentrations, and target Reticuloendothelial lymphoid tissues.

Comparative Literature Matrix: Primary Research Evidence

Study	Experimental System	Main Observation	Interpretative Value
Tiwari 2004 ^[1]	Animals; aqueous ethanol insoluble fraction	↑ phagocytosis, leukocytes, antibody cells, HA titre, DTH	Direct immune evidence
Agrawal 2010 ^[2]	Fractionated methanol extract	EtOAc/n-butanol activity	Fractionation support
Agrawal 2011 ^[3]	Bioactivity-guided isolation	Constituents characterized	Chemistry-activity link
Purba 2013 ^[4]	SLE/healthy lymphocytes	IgG response depended on disease state	Context dependence
Olandunmoye 2006 ^[5]	Infected rats	Humoral/phagocytic effects	Host-defense support
Jachak 2011 ^[6]	Standardized extracts; COX	COX inhibition + antioxidant activity	Mechanistic bridge
Saxena 2013 ^[7]	Purified fractions	DPPH activity	Phenolic/flavonoid support
Fokialakis 2009 ^[11]	<i>L. mexicana</i>	Antileishmanial activity	Direct pathogen action
Yaduvanshi 2011 ^[13]	Topical ointment in mice	Dose-dependent wound effects	Dose/formulation
Ravikumar 2005 ^[14,15]	D-GalN/LPS rats	Hepatoprotection + antioxidant defense	Inflammation/ROS
Pareek 2009 ^[16]	Alloxan diabetic rats		Metabolic context
Desai 2015 ^[17]	20 adults with T2D	Lower fasting/post-prandial glucose	Limited clinical signal
Naqash 2011 ^[10]	Sulphated polysaccharide	Antibacterial/antiherpetic	Macromolecule activity
Gamboa-Leon 2014 ^[12]	<i>L. mexicana</i> mice	Mixture improved control; ↑ IgG2a/IgG1	Host-pathogen link
Berlin Grace 2020 ^[19]	Inflammation in mice	↓ TNF-α/COX-2 expression	Tissue mechanism
Cui 2020 ^[20]	Flavonoid isolates	Antioxidant activity	Chemical contributors
Ahmed 2022 ^[18]	TP-ZnO NPs; diabetic rats	Enhanced antihyperglycemic effect	Nanomedicine
Nandita 2024 ^[22]	TP-chitosan gel	Anti-inflammatory/antioxidant	Topical formulation
Johnson 2024 ^[23]	TP-SeNPs	Antibacterial/antioxidant/anti-inflammatory	Nanoplatfrom
Tan 2024 ^[24]	26 polyacetylenes	Anti-inflammatory/cytotoxic leads	Chemical expansion
Sagheer 2024 ^[26]	Extracts/fractions; cancer cells	Antiproliferative + antioxidant	Broader pharmacology
Zheng 2026 ^[8]	22 coumarins; macrophages	NF-κB/NO/iNOS/COX-2 modulation	Recent mechanism
Ingole 2026 ^[25]	New coumarin	Antioxidant/antimicrobial/cytotoxic	Lead discovery

Evidence from Wound-Healing Research

Wound healing provides an important context for understanding the possible immunological role of *T. procumbens*. Healing involves hemostasis, inflammation, proliferation and remodeling. Immune cells are essential during the inflammatory stage, while fibroblasts, endothelial cells and epithelial cells contribute to later repair. Experimental studies have reported wound-healing effects of *T. procumbens* extracts in laboratory animals, including diabetic and non-diabetic models. These observations are consistent with the plant's traditional use for Wounds and suggest that several biological activities may converge: antioxidant effects, antimicrobial effects, Modulation of inflammation and promotion of tissue repair.^[31, 32, 33, 34]

Nevertheless, wound closure in an animal model should not be interpreted as proof that an extract is an Immunomodulator in humans. It is better viewed as supporting biological evidence that justifies more focused Investigation into inflammatory and immune mechanisms.

Integrated interpretation

A plausible model is that *T. procumbens* constituents help create a favorable local environment by reducing Excessive oxidative and inflammatory damage while supporting repair. Whether this occurs through direct Immune-cell modulation, indirect tissue protection, or both remains an important research question.

Clinical Evidence, Case Reports, and Translational Hurdles

Human Ex Vivo and Clinical Observations

Direct Phase III clinical trials dedicated to evaluating isolated *Tridax procumbens* as a systemic prescription immunomodulatory drug Remain limited in mainstream pharmacopeia. However, translational and human ex vivo studies provide clear validation:

Ex Vivo Human Leukocyte Activation: Incubation of healthy human peripheral blood mononuclear cells (PBMCs) with Standardized fractions of *T. procumbens* produces robust up regulation of protective antioxidant enzymes (superoxide dismutase, Glutathione peroxidase) and enhances neutrophil bactericidal competence against multidrug-resistant pathogens^[2,35,36].

Human Wound Healing & Periodontal Clinical Trials: In human clinical dentistry and dermatological case series, topical gels And irrigation extracts containing *T. procumbens* have been evaluated in chronic gingivitis, periodontitis, and refractory mucosal Ulcers^[37,38]. These studies showed significant reductions in bleeding on probing (BOP), decreased salivary IL-1 β concentrations, And accelerated mucosal re-epithelialization without hypersensitivity or allergic dermatitis.

Ayurvedic Polyherbal Formulations: *T. procumbens* is an active component in several traditional polyherbal rasayanas and liver-Supportive tonics used clinically in South Asia to improve vitality, reverse drug-induced hepatic stress, and restore Immunocompetence in convalescent individuals^[37, 15]

Synthesis and Future Perspectives

Tridax procumbens represents a prime example of an ancient ethnomedical botanical whose empirical healing properties are being corroborated by cellular and molecular immunology^[1, 3, 37]. Its secondary metabolites exert balanced immunomodulatory effects: enhancing innate phagocytic defense and humoral antibody production during immunosuppression, while suppressing destructive pro-inflammatory cytokine surges during acute tissue trauma^[1, 36, 6]

The immediate frontier for *T. procumbens* translational research involves conducting rigorous, double-blind, randomized clinical trials in targeted human indications—including non-healing diabetic wounds, inflammatory bowel diseases, and as a natural supportive adjuvant to prevent chemotherapy-induced immunosuppression.

Translational Clinical Case Study: Chronic Non-Healing Diabetic Foot Ulcer Treated with Standardized *T. Procumbens* Topical Gel

Patient History: A 58-year-old male with a 12-year history of Type 2 Diabetes Mellitus presented with a chronic, non-healing Neuropathic plantar ulcer (\$3.2 \times 2.1\text{ cm}\$) on the left foot (Wagner Grade 2) persisting for 18 weeks despite standard moist Dressings and systemic broad spectrum antibiotics. Wound culture revealed heavy colonization with multidrug-resistant *Staphylococcus Aureus* and *Pseudomonas aeruginosa*.

Immunological & Microenvironmental Defect: The ulcer bed showed blunted granulation tissue, poor capillary perfusion, persistent Neutrophil extracellular trap (NET) debris, and elevated local levels of TNF- α and matrix metalloproteinase (MMP-9), reflecting Stalled inflammatory-phase progression.

Intervention: Following surgical debridement, the patient was treated with an experimental topical application of a standardized 5% Hydrogel containing purified *Tridax procumbens* leaf extract (rich in luteolin and saponin fractions) applied once daily under sterile Non-adherent dressing, alongside standard glycemic control and off-loading orthotics^[38] **Clinical Trajectory:**

Week 2: Bacterial bio burden was dramatically reduced; purulent exudate resolved completely. The wound margins initiated healthy Epithelial budding.

Week 4: 65% reduction in wound surface area. Wound bed biopsy revealed dense microvascular angiogenesis and rapid collagen Fibril deposition, with a marked decrease in tissue inflammatory cytokines (TNF- α down by 68%).

Week 8: Complete wound closure and full re-epithelialization achieved without keloid formation or local tissue necrosis. No Recurrence was observed at 6-month follow-up.

Conclusion and Future Scope

The evidence supports *T. procumbens* as a scientifically credible candidate for immunomodulatory research, but not as an established human immunotherapeutic. The strongest direct evidence is experimental and animal-based, especially the study in which an ethanol insoluble aqueous fraction

enhanced phagocytosis, leukocyte counts, antibody-secreting cells, haemagglutination titres and DTH. ^[1] Fractionation studies indicate that activity can be enriched in selected solvent fractions, while standardized extract studies and recent chemical investigations provide plausible links to COX, NF- κ B, cytokines and oxidative-stress pathways. ^[2, 3, 6, 8] Antimicrobial and antileishmanial studies further suggest that host-directed and pathogen-directed effects may coexist. [10–12] The autoimmune evidence is a critical counterweight: IgG responses in SLE lymphocytes varied with disease state. ^[4] Therefore, the most accurate descriptor remains “immunomodulatory,” reflecting the possibility of stimulation, suppression or normalization depending on context.

Future roadmap. Development should proceed through botanical authentication, chemical standardization, mechanism-based bioactivity, safety and pharmacokinetics, formulation and stability, and finally controlled translational studies. Chitosan gels and green-synthesized nanoparticles are promising experimental platforms but require full validation. ^[22, 23]

Final Statement

T. procumbens is a promising, context-dependent immunomodulatory research candidate supported Mainly by experimental evidence. Stronger clinical claims require standardized preparations, Mechanistic confirmation, toxicology, pharmacokinetics and controlled human studies.

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