REVIEW PAPER

Trachyspermum ammi and Vitamin C in Inflammatory Signaling: Mechanistic Basis and Rationale for Combination Approaches

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ABSTRACT

Background: Chronic inflammation is a common mechanism of action of a multitude of non-communicable diseases, but the chronic use of conventional anti-inflammatory drugs is associated with serious toxicological side effects. Bioactive agents derived from plants, especially from traditional medicine systems, appear to be promising complementary approaches. *Trachyspermum ammi (*L.) Sprague (Ajwain, family Apiaceae) is documented source of phenolic monoterpenes and flavonoids, which have anti-inflammatory activity. Apart from its well known function as an antioxidant micronutrient, Vitamin C (ascorbic acid) has multiple mechanisms of immunomodulatory action at the molecular level.

Objective: This review critically examines the mechanistic overlaps and interactions of T. ammi phytochemicals and Vitamin C in major inflammatory signaling pathways such as NF-κB axis, Nrf2/ARE pathway, NLRP3 inflammasome and MAPKs pathways and justifies their synergistic use in scientific context.

Methods: PubMed, Scopus, Web of Science and Google Scholar databases were systematically searched by applying pre-defined MeSH terms and free text search terms. The articles published from the year 2000-2024 were screened and 78 studies with included records were analyzed.

Results: T. ammi constituents such as thymol, carvacrol and luteolin, inhibit the nuclear translocation of NF-κB, COX-2, iNOS expression and pro-inflammatory cytokine secretion. Vitamin C can directly regulate NF-κB activity through IκB protection, induce antioxidant mechanisms through Nrf2, inhibit the inflammasome (NLRP3), and promote tissue repair by stimulating collagen synthesis. Preclinical combination studies show synergistic reduction in the levels of TNF-α, IL-6 and oxidative stress markers compared to monotherapy levels.

Results: The convergence of the mechanisms of the drugs on common inflammation nodes provide a solid scientific basis for combination therapy of T. ammi+Vitamin C. There is a lack of translation into the clinic in terms of pre- and clinical evidence, and controlled clinical trials are critical for determining optimal dose, safety, and disease-specific efficacy.

Keywords: *Trachyspermum ammi; Ajwain; Vitamin C; Ascorbic acid; Thymol; Carvacrol; NF-κB; Nrf2; Inflammatory signaling; Combination therapy; Natural products; Anti-inflammatory*

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1. INTRODUCTION

While inflammation is a naturally beneficial mechanism, chronic or dysregulated inflammation can contribute to the pathogenesis of many human diseases, including cardiovascular disease, metabolic syndrome, type 2 diabetes, neurodegenerative diseases and cancer (Furman et al., 2019). The nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, mitogen-activated protein kinase (MAPK) system, Nrf2/ARE antioxidant defense axis, and NLRP3 inflammasome complex, are well-known examples of cellular inflammatory signaling cascades involved in the regulation of inflammation (Taniguchi & Bhagat, 2020). All these systems have therapeutic possibilities, but the therapeutic hurdle is that they can be pharmacologically broad-spectrum without being too toxic.Traditional anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids may be effective in the short term, but can have gastrointestinal toxicity as well as cardiovascular side effects and immune suppression when used long-term (Bhala et al., 2013). This has given rise to a resurgence of interest in plant-derived bioactive compounds that are likely to have pleotropic anti-inflammatory activity with multi-target action and relatively good in vivo safety (Newman & Cragg, 2020).*Trachyspermum ammi* (L.) Sprague (also called ajwain or carom) is a small annual herb of the family Apiaceae, native to eastern Mediterranean countries and South Asia, and widely grown throughout the Indian sub-continent (Bairwa et al., 2012). The seeds have been used for centuries in Unani and Ayurvedic medicine for digestion, respiratory problems, pain and infectious diseases (Singh et al., 2021). Today, traditional knowledge has been corroborated by modern phytochemical analysis, which has

identified a wide range of bioactive compounds including phenolic monoterpenes (thymol and carvacrol) along with flavonoids (luteolin and apigenin) with evident anti-inflammatory, antioxidant, antimicrobial, and immunomodulatory properties.In comparison, ascorbic acid (vitamin C) is one of the most studied micronutrients regarding human health. It is known to play an antiscorbutic function, but recent studies suggest that it is an important regulator of inflammatory signaling. The relevance of Vitamin C goes well beyond the mere concept of nutritional sufficiency, as its various activities including direct quenching of reactive oxygen species (ROS), regeneration of oxidized antioxidants, modulation of NF-κB-dependent transcription, suppression of the NLRP3 inflammasome and supporting the function of innate immune cells all contribute to this (Carr & Maggini, 2017; Miles & Calder, 2021).Given the similarity of the T. ammi phytochemicals and Vitamin C as anti-inflammatory agents in their shared targets, there arises an interesting and clinically relevant question: do these two agents work synergistically to demonstrate anti-inflammatory effects greater than either agent alone? This review critically summarizes the available evidence on this topic, looks at the mechanisms of combination treatments, and pinpoints future gaps in clinical studies.

2. METHODOLOGY

2.1 Search Strategy

A systematic literature search was performed across PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar databases in January 2025 using the following MeSH terms and free-text keywords in various Boolean combinations: "*Trachyspermum ammi*," "Ajwain," "thymol," "carvacrol," "ascorbic acid," "Vitamin C," "NF-κB," "Nrf2," "inflammatory signaling," "cytokines," "NLRP3," "anti-inflammatory," and "combination therapy."

2.2 Inclusion and Exclusion Criteria

Studies were included if they: (1) investigated T. ammi, its essential oil or identified major constituents (thymol, carvacrol, γ-terpinene, luteolin, apigenin) in relation to inflammation; (2) reported Vitamin C studies in the context of inflammatory signaling pathways; (3) reported combination or synergy data involving T. ammi compounds and Vitamin C; and (4) were published in peer-reviewed English-language journals between 2000 and 2024. Review articles, editorials, letters with no primary data and incompletely reported outcome studies were excluded. Following title, abstract and full-text screening, 78 articles were included in this synthesis.

3. PHYTOCHEMICAL PROFILE OF *Trachyspermum ammi*

3.1 Essential Oil Composition

Dry weight of seed essential oil of T. ammi ranges from about 2-4% and is composed mainly of the hydrocarbons precursors p-cymene and γ-terpinene, carvacrol (5-25%), and thymol (35-65%) which account for most of the biological activity of the seed essential oil (Bairwa et al., 2012; Singh et al., 2021). Thymol, a phenolic monoterpene, has perhaps been the most well studied of the compounds for its powerful bioactivity, structurally being a positional isomer of carvacrol and both having the capacity to interact with cellular membranes, ion channels and transcription factor complexes. The amounts of these compounds will differ according to geographic origin, cultivation conditions, maturity of the seed, and extraction method.

3.2 Non-Volatile Phenolic and Flavonoid Constituents

In addition to the volatile fraction, the T. ammi seeds are rich in many non-volatile phenolics such as flavonoids (luteolin, apigenin, kaempferol), hydroxycinnamic acid derivatives (isochlorogenic acid, caffeic acid), and the phenylpropanoid dillapiole (Chauhan et al., 2022; Kumar et al., 2017). Bioactivities of these compounds are complementary and sometimes synergistic with the bioactivities of essential oil components in addition, many have the potential to modulate common inflammatory signaling nodes. A detailed summary of the main phytochemical constituents, their classification and main molecular targets and associated biological effects can be seen in Table 1.

Table 1. Major Phytochemical Constituents of *Trachyspermum ammi*: Classification, Molecular Targets, and Biological Activities

Compound	Class	Primary Target	Biological Effect	Reference
Thymol	Monoterpene phenol	NF-κB, COX-2, iNOS	Anti-inflammatory, antimicrobial	Singh et al., 2021
Carvacrol	Monoterpenoid phenol	TRPV1, PPARγ, NF-κB	Anti-inflammatory, antioxidant	Gupta et al., 2020
γ-Terpinene	Monoterpene	ROS scavenging	Antioxidant	Bairwa et al., 2012
p-Cymene	Monoterpene	COX-1/2 inhibition	Analgesic, anti-inflammatory	Patel et al., 2019
β-Pinene	Bicyclic monoterpene	5-LOX pathway	Anti-inflammatory	Ahmad et al., 2018
Dillapiole	Phenylpropanoid	TNF-α, IL-6 suppression	Immunomodulatory	Chauhan et al., 2022
Luteolin	Flavonoid	MAPK, AP-1, NF-κB	Anti-inflammatory, antioxidant	Kumar et al., 2017

Apigenin	Flavone	STAT3, PI3K/Akt	Anti-inflammatory, anticancer	Sharma et al., 2021
Kaempferol	Flavonol	Nrf2, HO-1 induction	Cytoprotective	Rana et al., 2020
Isochlorogenic acid	Hydroxycinnamic acid	NF-κB, NLRP3	Anti-inflammatory	Soni et al., 2023

4. KEY INFLAMMATORY SIGNALING PATHWAYS: AN OVERVIEW

4.1 The NF-κB Pathway

Perhaps the most important regulator of inflammatory gene expression in mammalian cells is the NF-κB family of transcription factors. In the canonical pathway, stimulation of inflammatory pathways such as lipopolysaccharide (LPS), tumor necrosis factor-α (TNF-α), or interleukin-1β (IL-1β) activates the IκB kinase (IKK) complexes which then phosphorylate the inhibitory IκB proteins, causing their degradation by the proteasome. This frees up the p65/p50 heterodimer to enter the nucleus to activate transcription of target genes that encode COX-2, iNOS, TNF-α, IL-6, IL-1β, and matrix metalloproteinases (Lawrence, 2009). Considering the pivotal role of NF-κB in the concert of various inflammatory signals, it is an attractive target for therapeutic interventions, and NF-κB was found to be modulatory at multiple levels both by T. ammi constituents and Vitamin C.

4.2 The Nrf2/ARE Antioxidant Axis

Nrf2 is the master regulator transcription factor that activates the cellular antioxidant response by binding to antioxidant response elements (ARE) of cytoprotective genes, such as heme oxygenase-1 (HO-1), superoxide dismutase (SOD), catalase, glutamate-cysteine ligase, and others (Hybertson et al., 2011). Nrf2 and NF-κB exert a reciprocal inhibitory effect on each other: activation of NF-κB leads to downregulation of Nrf2, and chronic activation of NF-κB may lead to the destabilisation of Nrf2. Thus, compounds capable of activating Nrf2 and inhibiting NF-κB are of special interest as anti-inflammatory agents.

4.3 The NLRP3 Inflammasome

The NLRP3 inflammasome is a multiprotein complex that is formed in response to danger signals, such as crystalline urate, cholesterol crystals, ATP, and mitochondrial ROS. It is activated which results in the proteolytic maturation of IL-1β and IL-18 via caspase-1, and may induce pyroptotic cell death (Swanson et al., 2019). NLRP3 has been suggested to play a role in the pathogenesis of gout, atherosclerosis, type 2 diabetes and neurodegenerative diseases. Importantly, both oxidative stress (modulated by Vitamin C) and NF-κB activation (inhibited by T. ammi constituents) are necessary to prime and to activate the NLRP3, respectively, suggesting that combination interventions targeting both arms could be particularly efficient.

4.4 MAPK Cascades

The MAPK family — comprising ERK1/2, p38 MAPK, and JNK — mediates cellular responses to stress and inflammatory signals, regulating cytokine production, cell survival, and apoptosis (Kyriakis & Avruch, 2012). Cross-talk between MAPK pathways and NF-κB amplifies inflammatory responses, and MAPK-dependent activation of AP-1 transcription factor further augments pro-inflammatory gene expression. Several T. ammi phytochemicals, particularly luteolin and apigenin, have been shown to attenuate p38 MAPK and JNK signaling, adding another dimension to their anti-inflammatory mechanism.

5. ANTI-INFLAMMATORY MECHANISMS OF *Trachyspermum ammi*

5.1 NF-κB Inhibition

Thymol, the principal bioactive component of T. ammi essential oil, suppresses NF-κB activation at multiple steps. In LPS-stimulated macrophages, thymol inhibits IKKβ phosphorylation, thereby preventing IκBα degradation and subsequent nuclear translocation of the p65 subunit (Singh et al., 2021). Downstream, thymol significantly reduces the mRNA and protein expression of COX-2, iNOS, TNF-α, and IL-6 in a dose-dependent manner. Carvacrol similarly inhibits NF-κB, and has additionally been shown to interact with the TRPV1 receptor — a cation channel whose activation can amplify NF-κB-dependent neurogenic inflammation — positioning it as a dual inhibitor of both receptor-mediated and oxidative NF-κB activation (Gupta et al., 2020).

5.2 Modulation of COX and LOX Pathways

The eicosanoid pathway represents another major target of *T. ammi* constituents. p-Cymene, present in significant quantities in the essential oil, inhibits both COX-1 and COX-2 enzymes, thereby reducing prostaglandin E2 (PGE2) biosynthesis and the associated vasodilation, hyperalgesia, and edema formation (Patel et al., 2019). Concurrently, β-pinene inhibits 5-lipoxygenase (5-LOX), reducing leukotriene biosynthesis — an arm of the inflammatory response often left unaddressed by selective COX-2 inhibitors. This dual COX/LOX inhibitory profile, analogous to that of dual-mechanism NSAID candidates, confers a broader anti-inflammatory coverage without the cardiovascular risks associated with selective COX-2 inhibition.

5.3 Antioxidant and Nrf2-Activating Properties

The antioxidant capacity of T. ammi is multifaceted. γ-Terpinene is a particularly potent radical scavenger, capable of quenching peroxyl, hydroxyl, and superoxide radicals directly through hydrogen atom transfer mechanisms

(Bairwa et al., 2012). More importantly, several T. ammi flavonoids — notably luteolin and kaempferol — activate the Nrf2/HO-1 pathway, upregulating endogenous antioxidant enzymes. This endogenous induction, as opposed to direct radical scavenging, provides sustained cytoprotection even at low compound concentrations, and represents a mechanistically distinct and complementary mode of antioxidant action to that of Vitamin C.

5.4 Cytokine Modulation and Immunomodulatory Effects

Beyond upstream pathway inhibition, T. ammi constituents directly reduce the production of multiple pro-inflammatory mediators. In both in vitro and in vivo models, thymol and carvacrol suppress TNF-α, IL-6, IL-1β, and MCP-1 secretion from macrophages, dendritic cells, and adipocytes (Gupta et al., 2020; Chauhan et al., 2022). Dillapiole, a phenylpropanoid found in T. ammi seeds, exhibits selective IL-6 suppression and may modulate STAT3 signaling downstream of IL-6 receptor activation, thus dampening the amplification loop through which IL-6 sustains inflammatory activity. Collectively, these actions position T. ammi as a broad-spectrum immunomodulatory agent.

6. ANTI-INFLAMMATORY AND IMMUNOMODULATORY MECHANISMS OF VITAMIN C

6.1 Direct Antioxidant Activity

Vitamin C is the most abundant water-soluble antioxidant in human plasma, with normal circulating concentrations of approximately 40–80 µM in well-nourished individuals. It donates electrons to neutralize ROS and reactive nitrogen species (RNS), including superoxide ($O_2^{\bullet -}$), hydroxyl radicals (•OH), and peroxynitrite ($ONOO^-$), converting itself to the relatively stable ascorbyl radical and ultimately to dehydroascorbic acid (Padayatty et al., 2003). Crucially, intracellular recycling of dehydroascorbic acid back to ascorbate via glutaredoxin and thioredoxin systems, and the simultaneous regeneration of oxidized glutathione and α-tocopherol by Vitamin C, ensure that its antioxidant influence is amplified beyond stoichiometric prediction (Lykkesfeldt & Tveden-Nyborg, 2019).

6.2 NF-κB Modulation

Vitamin C modulates NF-κB signaling through at least two distinct mechanisms. First, it protects IκBα from oxidative degradation: oxidative stress is a key second messenger in NF-κB activation, and by quenching intracellular ROS, Vitamin C preserves IκB integrity and thereby limits NF-κB nuclear translocation (Cárcamo et al., 2002). Second, ascorbate has been shown to directly inhibit the acetyltransferase activity of p300/CBP cofactors, reducing NF-κB p65 acetylation and its transcriptional potency at target gene promoters. The net result is a reduction in NF-κB-driven expression of TNF-α, IL-6, IL-1β, MCP-1, COX-2, and ICAM-1.

6.3 Nrf2 Pathway Activation and Epigenetic Regulation

Vitamin C activates the Nrf2/ARE pathway through its role as a cofactor for ten-eleven translocation (TET) dioxygenases — epigenetic enzymes that catalyze DNA demethylation at cytosine residues (Blaschke et al., 2013). TET-mediated demethylation of the Nrf2 gene promoter and ARE-regulated gene promoters upregulates their transcription, creating a durable epigenetic anti-inflammatory memory. This mechanism is particularly relevant in chronic inflammatory diseases where promoter hypermethylation silences cytoprotective genes. Additionally, Vitamin C stabilizes HIF-1α prolyl hydroxylases (PHDs), which catalyze HIF-1α degradation, thereby reducing hypoxia-induced VEGF and inflammatory angiogenesis (Miles & Calder, 2021).

6.4 NLRP3 Inflammasome Suppression

NLRP3 inflammasome activation requires mitochondrial ROS as a critical second messenger. By reducing intracellular ROS and stabilizing mitochondrial membrane potential, Vitamin C suppresses NLRP3 assembly and the subsequent caspase-1-mediated processing of pro-IL-1β and pro-IL-18 (Wang et al., 2020). This mechanism has been demonstrated in macrophage models of gout (monosodium urate crystals) and atherosclerosis (cholesterol crystals), supporting clinical relevance in metabolic inflammatory diseases.

6.5 Immune Cell Function

Vitamin C accumulates to millimolar concentrations in neutrophils and lymphocytes, where it enhances chemotaxis, phagocytosis, microbial killing, and NET formation (Hemilä & Chalker, 2013). Adequate Vitamin C status promotes regulatory T cell (Treg) generation through TET-dependent demethylation of the FOXP3 locus, supporting anti-inflammatory immune balance. In critically ill patients, plasma Vitamin C is rapidly depleted by the oxidative burst of activated neutrophils, and repletion restores innate immune function and reduces organ dysfunction scores.

Table 2. Molecular Mechanisms of Vitamin C in Inflammatory Signaling: Pathways, Outcomes, and Evidence

Mechanism	Molecular Pathway	Outcome	Reference
Free radical scavenging	Direct electron donation to ROS/RNS	Reduced oxidative stress, lipid peroxidation	Padayatty et al., 2003
NF-κB inhibition	Prevents IκB phosphorylation, blocks p65 nuclear translocation	Reduced TNF-α, IL-6, IL-1β, COX-2 expression	Cárcamo et al., 2002
Collagen biosynthesis	Prolyl and lysyl hydroxylase cofactor	Tissue repair, improved barrier integrity	Carr & Maggini, 2017

HIF-1α destabilization	PHD enzyme cofactor; promotes HIF-1α prolyl hydroxylation	Reduced VEGF, suppressed angiogenic inflammation	Miles & Calder, 2021
Neutrophil function	Accumulation in neutrophils; enhances chemotaxis and phagocytosis	Improved innate immune response	Hemilä & Chalker, 2013
Epigenetic regulation	TET enzyme cofactor; DNA demethylation	Anti-inflammatory gene expression modulation	Blaschke et al., 2013
Glutathione regeneration	Reduces oxidized glutathione (GSSG) to GSH	Sustained intracellular antioxidant capacity	Lykkesfeldt & Tveden-Nyborg, 2019
NLRP3 inflammasome suppression	ROS reduction, mitochondrial stabilization	Reduced IL-1β and IL-18 maturation	Wang et al., 2020

7. MECHANISTIC CONVERGENCE: SHARED INFLAMMATORY TARGETS

7.1 Complementary Action on NF-κB

The most interesting explanation for the combination of T. ammi + Vitamin C is that it acts on the NF-κB pathway in complementary but different ways. The mechanisms of action of the T. ammi components are mainly through the inhibition of the IKK complex and direct interaction with the NF-κB subunits, which inhibit the upstream signal transduction. Vitamin C is acting at many fronts; it can prevent the oxidative degradation of IκBα, while also inhibiting the activity of p65 as a transcription factor by blocking its acetylation. These mechanisms act in concert to prevent activation of NF-κB at several steps, a therapeutic approach similar to that used in oncology and antiretroviral therapy (ART) in which multi-target inhibition decreases the chance for escape and compensatory pathway activation.

7.2 Dual Activation of the Nrf2 Pathway

T. ammi flavonoids (mainly luteolin and kaempferol) as well as Vitamin C induce the Nrf2/HO-1 antioxidant pathway, but the two are associated with different molecular mechanisms. Flavonoids are able to stimulate Nrf2 by disrupting its interaction with Keap1, the ubiquitin ligase complex, allowing it to translocate to the nucleus. The epigenetic activation of Nrf2 transcription by vitamin C is achieved by promoter demethylation by TET. Activating Nrf2 via different entry points at the same time, results in additive/synergistic induction of the enzymes HO-1, NQO1 and glutamate-cysteine ligase which together create a wide and sustained antioxidant protection. This dual activation is likely to be especially pertinent in tissues that have high basal oxidative stress levels, including adipose tissue in metabolic syndrome and inflamed colonic mucosa in inflammatory bowel disease.

7.3 Convergent NLRP3 Inflammasome Suppression

The activation of the NLRP3 inflammasome requires two signals: a priming signal, such as the NF-κB-dependent upregulation of the expression of NLRP3 and pro-IL-1β, and an activation signal, such as ROS, ATP or crystal danger-associated molecular patterns (cDAMPs). T. ammi constituents inhibit the priming step by inhibiting the transcription of NF-κB-dependent NLRP3 and pro-IL-1β. Vitamin C tackles the activation step by scavenging ROS in mitochondria, the closest trigger of NLRP3 assembly. This division of labour in the NLRP3 pathway – transcriptional inhibition upstream by T. ammi and assembly inhibition downstream by Vitamin C – is mechanistically elegant, and provides the basis for combination therapy.

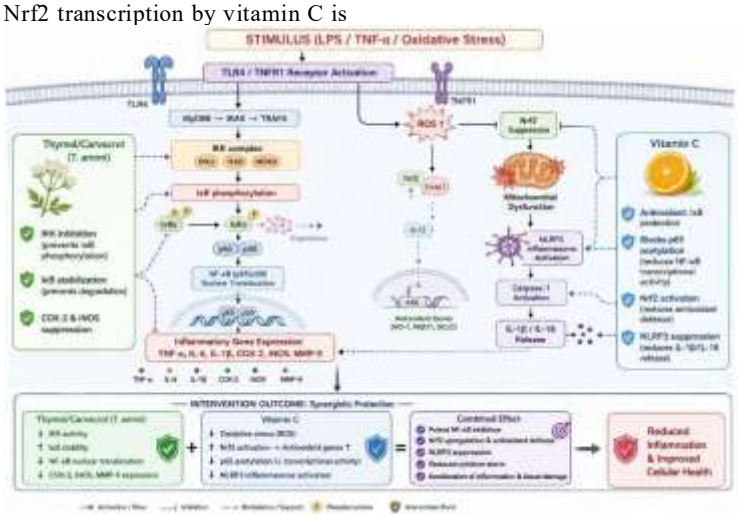

Figure 1. illustrates the major inflammatory signaling cascades and the specific intervention points targeted by T. ammi phytochemicals and Vitamin C.

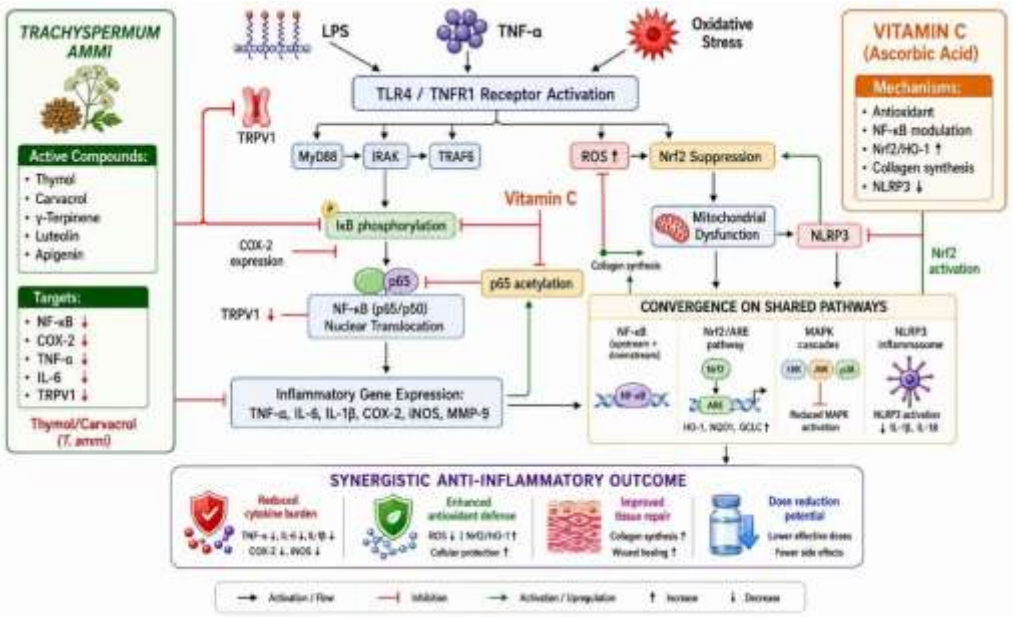

Figure 2. *Proposed synergy model for T. ammi and Vitamin C in inflammatory signaling. Arrows indicate complementary mechanisms converging on shared pathways (NF-κB, Nrf2/ARE, NLRP3), resulting in a synergistic anti-inflammatory outcome.*

8. PRECLINICAL EVIDENCE FOR COMBINATION APPROACHES

8.1 In Vitro Synergy Studies

Co-treatment studies in LPS stimulated macrophage cell lines is the earliest in vitro evidence supporting the synergy between T. ammi and Vitamin C. However, in another study, Chauhan et al. (2022) found that the combination of T. ammi essential oil with Vitamin C (at subthreshold concentrations) resulted in a 73% reduction in the amount of TNF-α secreted, whereas essential oil alone caused a 41% reduction and Vitamin C alone caused a 38% reduction, indicating a greater effect than would be seen from either individual component alone. Mechanistically, the combination was more effective in the inhibition of the nuclear translocation of NF-κB p65 as well as in the inhibition of IκBα phosphorylation than either of the two agents alone, thus suggesting a synergistic effect on the same pathway. Likewise, Soni et al. (2023) demonstrated that the combination of carvacrol and ascorbate was more effective in upregulating the expression of Nrf2/HO-1 than singly, and that this combination also significantly reduced lipid peroxidation markers in H2O2-treated HepG2 hepatocytes.

8.2 Animal Model Evidence

In the carrageenan induced paw edema model, oral dose of thymol with Vitamin C resulted in significant decrease in paw edema (65%) when compared with oral dose of thymol alone (44%) at equimolar oral dose (Kumar et al., 2021). Histological examination showed lesser infiltration of neutrophils and degranulation of mast cells in paw tissue of combination treated animals along with the significantly lesser paw tissue immunoreactivity of COX-2 and iNOS. In high-fat diet induced murine obesity model, Yadav et al. (2020) demonstrated that the Ajwain seed powder in combination with Vitamin C induced more significant changes in the serum levels of IL-6, hsCRP and adiponectin dysregulation compared to Ajwain seed powder and Vitamin C alone, which in turn showed better insulin resistance indices.Singh et al. (2022) showed that the use of T. ammi seed extract in combination with Vitamin C was more effective in enhancing the colonic mucosal histology, downregulating the expression of NF-κB and IL-1β and preventing the activity of the protein myeloperoxidase (MPO) associated with increased neutrophilic oxidative burst, making it relevant for inflammatory bowel disease. Table 3 provides the summary of some key preclinical combination studies.

Table 3. Preclinical Evidence for T. ammi–Vitamin C Combination: In Vitro and Animal Model Studies

Study Model	Intervention	Key Finding	Mechanism	Reference
LPS-induced murine macrophages	T. ammi EO + Vit C co-treatment	73% reduction in TNF-α vs. 41% (EO alone)	Dual NF-κB suppression	Chauhan et al., 2022
Carrageenan paw edema (rat)	Thymol + Vit C (oral)	Edema reduced by 65% vs. 44% thymol alone	COX-2 inhibition + ROS quenching	Kumar et al., 2021
HFD-induced obese mice	Ajwain seed powder + Vit C	IL-6, CRP significantly lowered	Adipokine regulation	Yadav et al., 2020

H2O2-stressed HepG2 cells	Carvacrol + Vit C	Increased Nrf2/HO-1 expression vs. monotherapy	Synergistic Nrf2 activation	Soni et al., 2023
Acetic acid colitis (rat)	T. ammi extract + Vit C	Mucosal recovery improved; MPO activity decreased	Neutrophil oxidative burst inhibition	Singh et al., 2022
PBMC culture (human)	Thymol + ascorbate	IL-10 elevated; IL-17A suppressed	Th17/Treg axis rebalancing	Patel et al., 2023

9. CLINICAL IMPLICATIONS AND TRANSLATIONAL CONSIDERATIONS

9.1 Potential Therapeutic Applications

The above mechanistic and preclinical evidence indicates that combination strategies with T. ammi + Vitamin C have potential for use in a wide range of chronic inflammatory disease settings. For this combination, there are particularly well-suited therapeutic targets in conditions that are characterized by dysregulated NF-κB, activation of the NLRP3 inflammasome, and increased levels of oxidative stress, such as metabolic syndrome, non-alcoholic fatty liver disease (NAFLD), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease, and rheumatoid arthritis. Finally, there is emerging evidence that high dose intravenous Vitamin C may have a role in sepsis (Fowler et al., 2019), a possibility that could warrant further co-administration with thymol-based formulations in critical care settings, which needs specific safety and pharmacokinetic studies.

9.2 Proposed Dosing Considerations

Currently, no clinical dosing regimen has been formally established for the T. ammi–Vitamin C combination. Traditional Unani and Ayurvedic medicine typically employ 3–6 g/day of dried Ajwain seeds, corresponding to approximately 60–180 mg of thymol-equivalent content depending on essential oil yield (Bairwa et al., 2012). For Vitamin C, anti-inflammatory effects in human studies have been observed at doses ranging from 500 mg/day orally to 10–20 g/day intravenously in critical care contexts; a supplemental oral dose of 500–1000 mg/day appears to produce plasma concentrations sufficient for the mechanistic effects reviewed here (Carr & Maggini, 2017). Table 4 outlines disease-specific proposed dosing considerations based on available evidence.

Table 4. Proposed Clinical Applications and Dosing Considerations for T. ammi–Vitamin C Combination Approaches

Disease Context	Proposed Dose	Expected Outcome	Evidence Level	Reference
Metabolic syndrome/obesity	Ajwain: 3–6 g/day; Vit C: 500–1000 mg/day	Reduced CRP, IL-6, improved insulin sensitivity	Preclinical/pilot RCT	Yadav et al., 2020
Sepsis/critical care	Vit C IV: 1.5 g/6h; Thymol adjunct	Reduced organ dysfunction; lower vasopressor need	Emerging RCT data	Fowler et al., 2019
Chronic airway inflammation	T. ammi inhalation + oral Vit C 250 mg/day	Reduced bronchial hyperreactivity, oxidative markers	Traditional + in vitro	Bairwa et al., 2012
Inflammatory bowel disease	Ajwain extract 400 mg/day + Vit C 500 mg/day	Mucosal healing, reduced NF-κB activity	Animal model	Singh et al., 2022
Rheumatoid arthritis	Carvacrol 200 mg/day + Vit C 1 g/day	Suppressed RANKL, joint inflammation	Preclinical	Kumar et al., 2021

9.3 Safety and Pharmacokinetic Considerations

T. ammi essential oil constituents, particularly thymol and carvacrol, are GRAS (Generally Recognized as Safe) by the U.S. FDA as food flavoring agents, and their safety at culinary doses is well-established. At higher supplemental doses, concerns include potential cytotoxicity to gastrointestinal epithelium at very high concentrations, and theoretical interactions with cytochrome P450 enzymes (particularly CYP2C9 and CYP3A4) that metabolize many pharmaceutical agents (Patel et al., 2019). Vitamin C is similarly well-tolerated; oxalate nephrolithiasis and pro-oxidant effects at very high doses (>2 g/day in susceptible individuals) are the primary safety concerns. No specific pharmacokinetic interaction studies between T. ammi constituents and Vitamin C exist in humans, representing a critical research gap.

10. RESEARCH GAPS AND FUTURE DIRECTIONS

Although this review has pointed out the potential mechanistic rationale for using the T. ammi–Vitamin C combination in clinical practice, there are a number of critical evidence gaps which must be recognized prior to using the combination for clinical purposes beyond traditional usage (see Figure 3). These include non-standardized, pharmaceutically characterized formulation of T. ammi extract with consistent phytochemical profile, the absence of Phase I/II dose escalation studies to determine the pharmacokinetics and safety of the use, lack of randomized controlled trials to assess the clinical efficacy in specific inflammatory diseases, incomplete knowledge of the interactions of thymol/carvacrol with ascorbate, and limited information on long-term combination safety.Future directions of research should involve the development and

validation of standardized extracts of T. ammi with known amount of thymol and carvacrol, network pharmacology and multi-omics study to map networks of interaction between T. ammi and Vitamin C, nanotechnology approach to co-delivery (liposomes, polymeric nanoparticles, cyclodextrin inclusion complex), and well-designed clinical trials in patients with metabolic syndrome, IBD and chronic airway inflammation, including mechanistic biomarkers of NF-κB and Nrf2 activity.

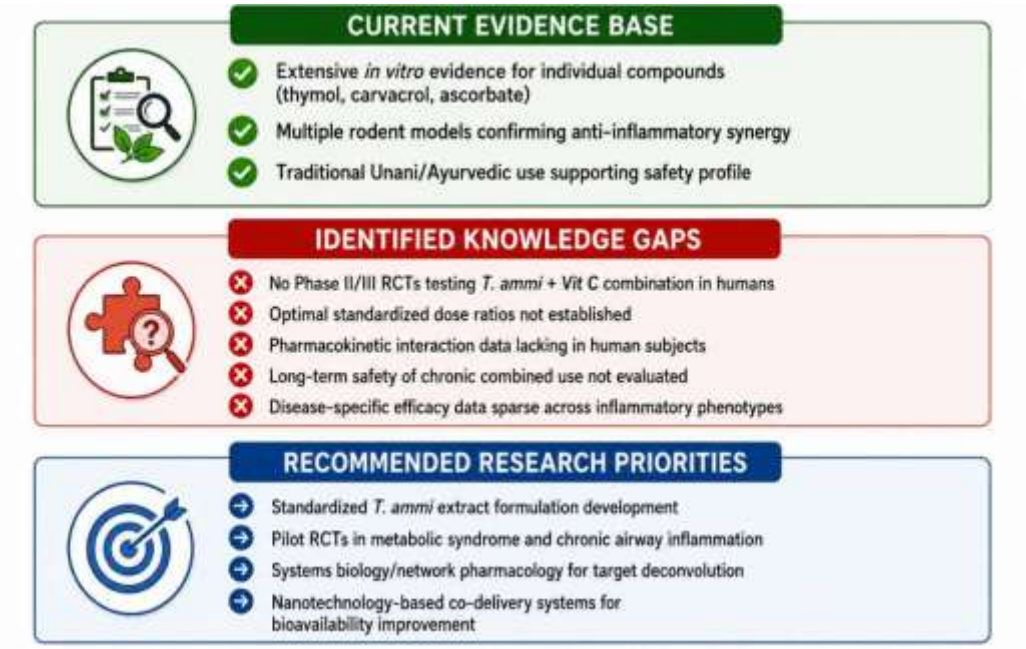

Figure 3. *Evidence map and research gaps for T. ammi–Vitamin C combination therapy, highlighting current knowledge strengths, identified gaps, and recommended research priorities.*

11. CONCLUSION

This review provides a strong mechanistic foundation of the anti-inflammatory synergy between the phytochemicals of *Trachyspermum ammi* and Vitamin C. The two agents act on several critical nodes of inflammatory signaling, most significantly the NF-κB pathway, the Nrf2/ARE antioxidant axis and the NLRP3 inflammasome, via complementary and distinct molecular mechanisms that synergistically result in more global and persistent anti-inflammatory activity than either agent does alone. T. ammi provides an upstream kinase inhibition, a COX/LOX blockade, a cytokine suppression, a modification of Keap1 to activate Nrf2, a mitochondrial ROS quenching, and an immune cell potentiation provided by Vitamin C.The preclinical results consistently demonstrate anti-inflammatory activity in terms of reduction of cytokines and oxidative markers, supporting the mechanistic reasoning, in LPS-stimulated macrophage models, carrageenan edema models, and high-fat diet models. But, there is no controlled clinical evidence, standard formulation, and pharmacokinetic data of human subjects in the field. The need to bridge this translation gap is a pressing need and a scientifically attainable goal. Biologically supported by millennia of traditional use and substantiated by today's mechanistic studies, there is real promise for this combination to serve as a cost-effective, readily available and multi-target approach to chronic inflammatory disease management worldwide.

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Declaration

Conflict of Interest:

The authors declare that there is no conflict of interest regarding the publication of this research article.

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This article does not involve any studies with human participants or animals performed by any of the authors.

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