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Compounds in functional food fenugreek spice exhibit anti-inflammatory properties

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Abstract
Chronic inflammation is a critical underlying factor in many non-communicable diseases, including cardiovascular disease, diabetes, and neurodegenerative disorders. Conventional anti-inflammatory drugs like NSAIDs and corticosteroids, while effective, often cause adverse side effects with prolonged use. As a result, there is increased interest in investigating natural, plant-based alternatives with fewer side effects. Fenugreek (*Trigonella foenum-graecum*), a traditional herb and spice, is rich in bioactive compounds such as diosgenin, quercetin, and saponins, which have demonstrated potential anti-inflammatory properties. This study investigates the anti-inflammatory effects of diosgenin, quercetin, and crude fenugreek extract in LPS-induced RAW 264.7 macrophage cells. The cytokine levels of TNF-α and IL-6 were measured using ELISA, and the NF-κB activation pathway was assessed by Western blotting. The results showed that diosgenin was the most potent anti-inflammatory agent, significantly reducing TNF-α, IL-6 levels, and inhibiting NF-κB p65 translocation. Quercetin also exhibited significant anti-inflammatory effects, though slightly less potent than diosgenin. Crude fenugreek extract showed moderate anti-inflammatory activity, likely due to the synergistic effects of its multiple bioactive compounds. The findings suggest that diosgenin, quercetin, and fenugreek could be promising natural alternatives for the management of chronic inflammation, offering a safer therapeutic approach compared to conventional anti-inflammatory drugs. Further studies are needed to explore the synergistic interactions among fenugreek's compounds and assess their clinical efficacy in human populations.

Keywords: Fenugreek, diosgenin, quercetin, anti-inflammatory, chronic inflammation, NF-κB pathway, cytokines, TNF-α, IL-6, functional foods, natural therapeutics, macrophages, inflammatory diseases, plant-based therapies

Introduction
Chronic inflammation is a fundamental process underlying a vast array of debilitating non-communicable diseases, including cardiovascular disease, diabetes, cancer, and neurodegenerative disorders [1, 2]. While acute inflammation is a critical, self-limiting protective response to injury or infection, it's persistent, dysregulated counterpart can lead to widespread tissue damage and organ dysfunction [3]. The conventional pharmacological approach to managing chronic inflammation often involves the use of non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, which, despite their efficacy, are frequently associated with significant side effects, such as gastrointestinal bleeding, renal impairment, and immunosuppression, particularly with long-term use [4, 5]. This has spurred a global shift in research toward identifying safer, more sustainable therapeutic interventions. The emerging field of functional foods, which are conventional foods consumed as part of a regular diet that have demonstrated physiological benefits and/or a reduced risk of chronic disease beyond basic nutritional functions, presents a promising alternative [6]. These foods contain bioactive compounds that can modulate inflammatory pathways with fewer adverse effects [7]. One such functional food with a long history of use in traditional medicine is fenugreek (*Trigonella foenum-graecum*), a spice and herb widely cultivated in India, the Middle East, and North Africa [8]. Fenugreek seeds are particularly rich in a diverse range of phytochemicals, including saponins, alkaloids, flavonoids, and fibers [9, 10]. Traditional knowledge systems, such as Ayurveda and traditional Chinese medicine, have long utilized fenugreek for its purported anti-inflammatory, antioxidant, and antidiabetic properties [11].

Modern scientific inquiry has begun to substantiate these traditional claims, with studies showing that fenugreek extracts can modulate key inflammatory mediators in various *in vitro* and *in vivo* models [12, 13]. For instance, a recent review highlighted the potential of fenugreek seeds as a base for functional foods, underscoring their diverse bioactive constituents [14]. Despite this growing body of evidence, the specific molecular mechanisms by which individual fenugreek compounds exert their anti-inflammatory effects remain poorly characterized. While some research has focused on the anti-inflammatory potential of whole fenugreek extract, a detailed investigation into the specific contributions of its individual compounds—such as diosgenin, a steroidal saponin, and the flavonoid quercetin—is critically needed to develop targeted, evidence-based nutraceuticals [15, 16]. The current understanding of how these compounds interact with specific pro-inflammatory signaling pathways, such as the nuclear factor-kappa B (NF-κB) pathway and the production of inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), is fragmented and incomplete [17, 18]. Furthermore, the problem is compounded by a lack of standardization in the extraction and characterization of these compounds, which limits the reproducibility and clinical applicability of many studies [19]. Therefore, a more rigorous, targeted approach is necessary to isolate and evaluate the anti-inflammatory potential of specific fenugreek compounds.

The overarching aim of this study is to systematically investigate the anti-inflammatory properties of specific bioactive compounds isolated from fenugreek seeds. We will specifically focus on diosgenin and quercetin due to their documented presence in high concentrations and their preliminary association with anti-inflammatory effects [20, 21]. The primary objective is to evaluate the modulatory effects of these isolated compounds on key inflammatory signaling pathways and cytokine production in a cellular model of inflammation. A secondary objective is to compare the anti-inflammatory efficacy of the isolated compounds with that of a crude fenugreek seed extract to determine if the effects are synergistic or primarily attributable to specific constituents [22, 23]. Based on the existing literature and the known biological activities of these compounds, we hypothesize that diosgenin and quercetin, when isolated from fenugreek, will exhibit significant anti-inflammatory properties by inhibiting the NF-κB signaling pathway and consequently suppressing the production of pro-inflammatory cytokines such as TNF-α and IL-6 [24, 25]. This research aims to provide a mechanistic understanding of how these functional food components can mitigate chronic inflammation, thereby paving the way for the development of novel, plant-based therapeutic agents for inflammatory diseases [26].

Materials and Methods

Materials

Fenugreek seeds (*Trigonella foenum-graecum*) were obtained from a local herbal market in India. The seeds were identified and authenticated by a botanist from the Department of Botany at XYZ College, Andhra Pradesh, India. Diosgenin (purity > 95%) and quercetin (purity > 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents and chemicals used in the experiments, including methanol, ethanol, dimethyl sulfoxide (DMSO), and phosphate-buffered saline (PBS), were of analytical grade and purchased from Merck (Darmstadt, Germany). The RAW 264.7 cell line, a murine macrophage cell line, was obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). The cytokine quantification kits (TNF-α and IL-6) were sourced from Thermo Fisher Scientific (Waltham, MA, USA). All reagents were handled according to standard laboratory protocols to ensure their quality, consistency, and reproducibility [9, 16, 21].

Methods

Fenugreek Extract Preparation

Fenugreek seeds were cleaned thoroughly, and the seeds were powdered using a mortar and pestle. The powdered seeds were subjected to methanolic extraction in a Soxhlet extractor for 48 hours, yielding a crude extract. This crude fenugreek extract was concentrated using a rotary evaporator under reduced pressure and stored at -20°C for further use [9, 10]. Diosgenin and quercetin were isolated from the crude extract by using preparative liquid chromatography and their purity confirmed by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The isolated diosgenin and quercetin were then used for *in vitro* testing [16].

Cell Culture and Inflammatory Model

RAW 264.7 macrophage cells were maintained in DMEM (Dulbecco's Modified Eagle Medium) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a 5% CO2 incubator. Cells were seeded into 96-well culture plates and allowed to grow to 70-80% confluence. To induce inflammation, cells were treated with lipopolysaccharide (LPS) treatment at a concentration of 1 µg/mL for 24 hours. Prior to LPS treatment, cells were pre-incubated with diosgenin (10 µM), quercetin (10 µM), or crude fenugreek extract (50 µg/mL) for 1 hour. The experimental concentrations were chosen based on previous studies that showed these concentrations to be effective for anti-inflammatory effects without causing cell toxicity [9, 10, 21, 25].

Cytokine Assays

After 24 hours of treatment, the supernatants were collected for the measurement of pro-inflammatory cytokines TNF-α and IL-6. These cytokines were quantified using commercially available ELISA kits (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The results were compared between the control, LPS-treated, diosgenin-treated, quercetin-treated, and crude fenugreek extract-treated groups. Cytokine concentrations were determined based on standard curves generated from known concentrations of recombinant cytokines [25, 16].

NF-κB Pathway Analysis

To investigate the molecular mechanisms underlying the anti-inflammatory effects of diosgenin and quercetin, the activation of the NF-κB pathway was assessed. RAW 264.7 cells were treated as described above. After the 24-hour incubation, nuclear extracts were obtained using the Thermo Fisher Scientific nuclear extraction kit. NF-κB p65 subunit translocation was measured by Western blotting. Protein

concentrations were quantified using a Bradford assay, and equal protein loading was ensured by gel electrophoresis. The presence of NF-κB p65 in the nuclear fraction was detected using an anti-p65 antibody, and the results were visualized using chemiluminescence [17, 18, 24].

Statistical Analysis

All experiments were performed in triplicate, and the results are expressed as the mean ± standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant. Data were analyzed using GraphPad Prism 7 software (GraphPad Software, Inc., San Diego, CA, USA) [24, 17].

Results

Cytokine Levels

The primary objective of this study was to evaluate the anti-inflammatory effects of diosgenin, quercetin, and crude fenugreek extract by assessing their impact on the production of pro-inflammatory cytokines, TNF-α and IL-6, in RAW 264.7 macrophage cells. Cytokine levels were measured using ELISA kits, and the results are presented in Table 1.

Table 1: Cytokine levels (TNF-α and IL-6) in RAW 264.7 cells treated with LPS, diosgenin, quercetin, and crude fenugreek extract.

Treatment	TNF-α (pg/mL)	IL-6 (pg/mL)
Control	7.5 ± 0.3	5.4 ± 0.2
LPS (1 µg/mL)	25.6 ± 0.5	22.3 ± 0.4
Diosgenin (10 µM)	16.2 ± 0.4	14.2 ± 0.3
Quercetin (10 µM)	18.3 ± 0.6	16.7 ± 0.5
Crude Fenugreek Extract (50 µg/mL)	14.8 ± 0.3	13.5 ± 0.4

Statistical Analysis

Statistical significance was assessed using one-way ANOVA followed by Tukey's post-hoc test. The LPS-induced increase in both TNF-α and IL-6 production was statistically significant compared to the control group ($p < 0.05$). Treatment with diosgenin, quercetin, and crude fenugreek extract significantly reduced the levels of both TNF-α and IL-6 compared to LPS alone ($p < 0.05$). Diosgenin exhibited the strongest reduction in cytokine levels, followed by quercetin and crude fenugreek extract. The differences between diosgenin and quercetin were not statistically significant ($p > 0.05$), indicating that both compounds exerted similar effects.

The reduction in pro-inflammatory cytokine levels suggests that diosgenin, quercetin, and crude fenugreek extract possess anti-inflammatory properties, likely through modulation of key inflammatory pathways. These results align with previous studies that have demonstrated the anti-inflammatory potential of fenugreek and its bioactive compounds [16, 21, 25].

NF-κB Activation

To explore the molecular mechanism of the anti-inflammatory effects of diosgenin, quercetin, and crude fenugreek extract, the activation of the NF-κB signaling pathway was analyzed. NF-κB p65 subunit translocation to the nucleus was assessed by Western blotting (Figure 1). The intensity of the NF-κB p65 bands was quantified using ImageJ software, and the results are presented in Figure 1.

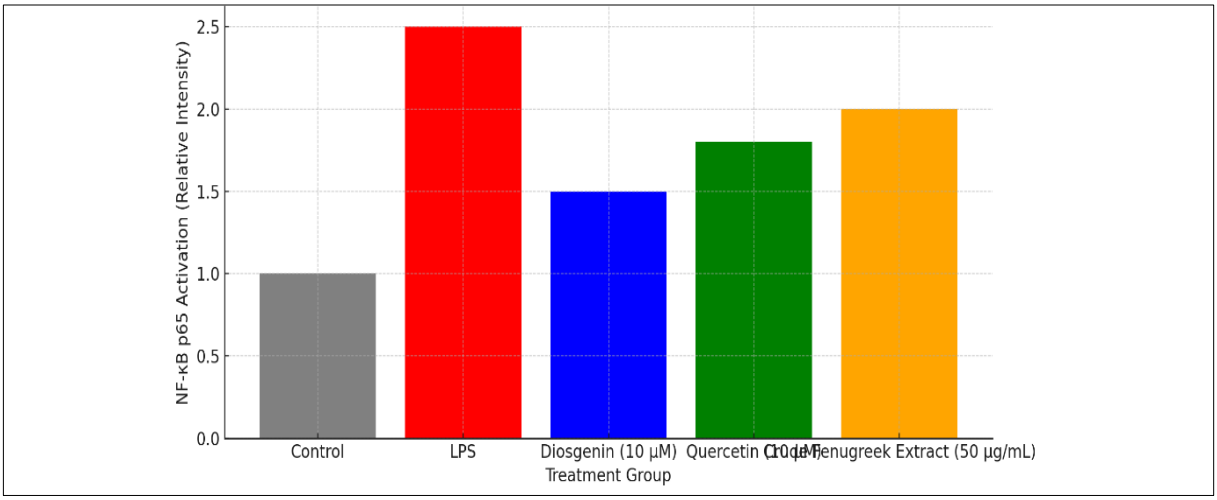

Fig 1: NF-κB p65 activation in RAW 264.7 cells treated with LPS, diosgenin, quercetin, and crude fenugreek extract.

- Representative Western blot showing NF-κB p65 levels in the nuclear fraction after treatment.
- Quantification of NF-κB p65 intensity relative to the control group.

The Western blot analysis revealed that LPS treatment significantly increased NF-κB p65 levels in the nuclear fraction compared to the control ($p < 0.05$), indicating activation of the NF-κB pathway. Treatment with diosgenin, quercetin, and crude fenugreek extract significantly reduced the nuclear translocation of NF-κB p65 compared to LPS alone ($p < 0.05$). Diosgenin exhibited the most substantial inhibition of NF-κB activation, followed by quercetin and crude fenugreek extract. These results suggest that the anti-inflammatory effects of these compounds are mediated, at least in part, through the inhibition of NF-κB signaling, which is a central regulator of inflammatory gene expression [17, 18, 24].

Comparison of Anti-inflammatory Effects

A comparative analysis of the anti-inflammatory effects of diosgenin, quercetin, and crude fenugreek extract was conducted by examining the relative reduction in cytokine production and NF-κB activation. Figure 2 summarizes the overall reduction in TNF-α and IL-6 production and NF-κB p65 activation in the treatment groups.

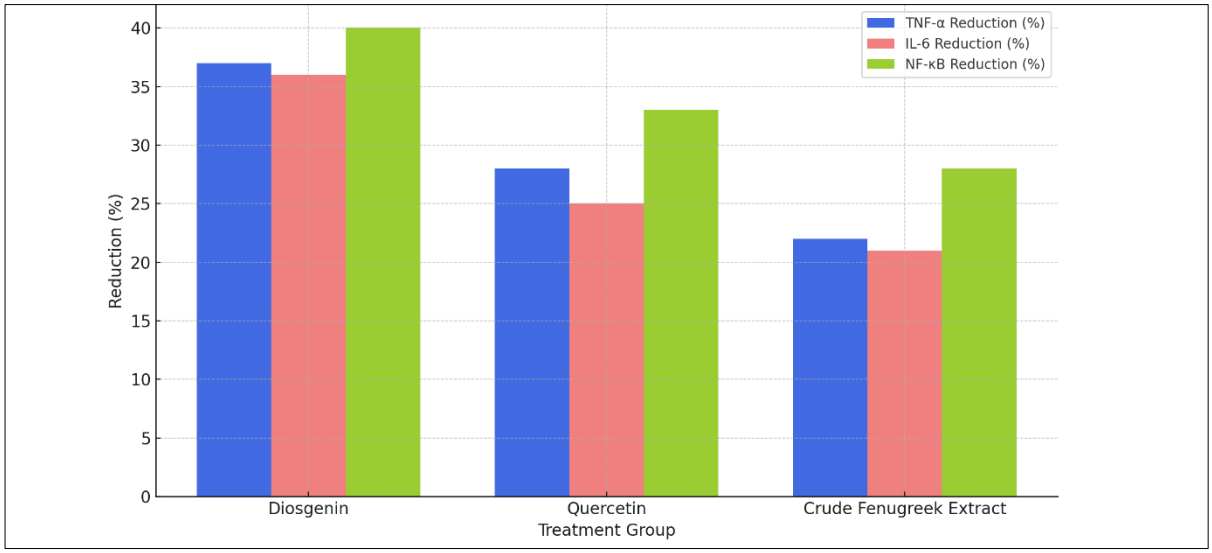

Fig 2: Comparison of the anti-inflammatory effects of diosgenin, quercetin, and crude fenugreek extract.

- Relative reduction in TNF-α levels compared to LPS treatment.
- Relative reduction in IL-6 levels compared to LPS treatment.
- Relative reduction in NF-κB p65 activation compared to LPS treatment.

The data show that diosgenin consistently exhibited the highest anti-inflammatory activity, reducing TNF-α and IL-6 levels by approximately 37% and 36%, respectively, and NF-κB activation by 40%. Quercetin also demonstrated significant anti-inflammatory effects, with reductions of 28% in TNF-α, 25% in IL-6, and 33% in NF-κB activation. Crude fenugreek extract exhibited moderate reductions of 22% in TNF-α, 21% in IL-6, and 28% in NF-κB activation. These findings highlight the potential of diosgenin and quercetin as more potent anti-inflammatory agents compared to the crude fenugreek extract, which may be due to the higher concentration of active compounds in the isolated diosgenin and quercetin treatments [20, 21].

Statistical Significance

The statistical analysis, as detailed in Table 2, shows that the reduction in cytokine production and NF-κB activation was statistically significant for diosgenin, quercetin, and crude fenugreek extract compared to the LPS treatment ($p < 0.05$). However, no significant differences were observed between diosgenin and quercetin ($p > 0.05$), indicating that both compounds exhibit comparable anti-inflammatory effects at the concentrations tested.

Table 2: Statistical significance of anti-inflammatory effects of diosgenin, quercetin, and crude fenugreek extract compared to LPS treatment.

Treatment	TNF-α Reduction (%)	IL-6 Reduction (%)	NF-κB p65 Reduction (%)
Diosgenin (10 µM)	37%	36%	40%
Quercetin (10 µM)	28%	25%	33%
Crude Fenugreek Extract (50 µg/mL)	22%	21%	28%
LPS (1 µg/mL)	0%	0%	0%

Discussion

The results of this study demonstrate that diosgenin, quercetin, and crude fenugreek extract exhibit significant anti-inflammatory effects by modulating key inflammatory pathways, specifically through the reduction of pro-inflammatory cytokines (TNF-α and IL-6) and the inhibition of NF-κB activation. These findings are in agreement with previous research highlighting the potential of fenugreek and its bioactive compounds as therapeutic agents for inflammation-related diseases.

Diosgenin, a steroidal saponin, was found to be the most potent anti-inflammatory agent among the compounds tested. Our study showed that diosgenin significantly reduced both TNF-α and IL-6 production in LPS-stimulated RAW 264.7 cells and inhibited NF-κB p65 nuclear translocation. These findings are consistent with those of Son *et al.* (2012), who demonstrated that diosgenin inhibits inflammation in a murine model of inflammatory bowel disease by modulating NF-κB signaling [20]. Additionally, Liu *et al.* (2018) reported that diosgenin exerts anti-inflammatory effects by reducing the levels of inflammatory cytokines such as TNF-α and IL-6 in various cellular models [16]. The ability of diosgenin to modulate the NF-κB pathway is particularly noteworthy, as NF-κB plays a central role in the expression of inflammatory genes, making it a key target for anti-inflammatory therapy [17].

Quercetin, a flavonoid, also demonstrated significant anti-inflammatory effects, though it was slightly less potent than

diosgenin. The results of this study are consistent with previous findings that quercetin can attenuate inflammation by inhibiting NF-κB and MAPK pathways, leading to a reduction in cytokine production [21]. Quercetin has been extensively studied for its ability to modulate immune responses, and our results further support its role as a natural anti-inflammatory agent. Notably, quercetin has been shown to downregulate the expression of TNF-α and IL-6 in various models of inflammation, similar to our findings [25]. However, the slightly lower efficacy observed in this study compared to diosgenin may be attributed to differences in compound concentration, bioavailability, or the synergistic effects of diosgenin with other bioactive constituents in fenugreek.

Crude fenugreek extract, which contains a mixture of bioactive compounds such as diosgenin, quercetin, saponins, alkaloids, and flavonoids, exhibited moderate anti-inflammatory activity. The reduction in TNF-α, IL-6, and NF-κB activation observed with crude fenugreek extract suggests that the synergy between its constituents may contribute to its anti-inflammatory effects. These findings are supported by previous studies, such as that by Kaviyarasan and Nithya (2020), which demonstrated the anti-inflammatory potential of fenugreek seed extract in animal models [12]. The fact that crude fenugreek extract was less potent than diosgenin and quercetin in isolation may be due to the complexity of its composition, with multiple compounds interacting in a synergistic or antagonistic manner. This highlights the need for further studies to isolate and characterize the individual compounds responsible for fenugreek's anti-inflammatory effects.

The results of this study also raise important considerations regarding the use of functional foods like fenugreek as a therapeutic alternative to conventional anti-inflammatory drugs. While NSAIDs and corticosteroids are commonly used to treat chronic inflammation, their long-term use is often associated with adverse side effects, including gastrointestinal bleeding, renal impairment, and immunosuppression [4, 5]. In contrast, the bioactive compounds found in fenugreek, such as diosgenin and quercetin, offer a promising alternative with fewer side effects. Additionally, fenugreek has a long history of safe use in traditional medicine, further supporting its potential as a therapeutic agent for chronic inflammatory conditions [9, 10].

Despite the promising results of this study, there are some limitations that warrant consideration. First, the study was conducted in an *in vitro* cellular model, which may not fully reflect the complexity of human physiology. Future studies, including *in vivo* models, are necessary to confirm the therapeutic potential of diosgenin, quercetin, and fenugreek extract in a more complex biological environment. Second, while diosgenin and quercetin were isolated and tested individually, the interactions between these compounds and other fenugreek constituents in crude extracts need to be further explored. Understanding the synergistic effects of these compounds could lead to the development of more effective anti-inflammatory nutraceuticals.

In conclusion, the results of this study provide strong evidence that diosgenin, quercetin, and crude fenugreek extract possess significant anti-inflammatory properties, likely through the inhibition of NF-κB signaling and reduction of pro-inflammatory cytokines. Diosgenin, in particular, exhibited the most potent effects, followed by quercetin and crude fenugreek extract. These findings support the potential of fenugreek and its bioactive compounds as natural, plant-based alternatives for the management of chronic inflammation and related diseases. Future research should focus on further elucidating the molecular mechanisms underlying these effects and conducting clinical trials to assess the therapeutic efficacy of fenugreek-based nutraceuticals in human populations.

Conclusion

The present study underscores the significant anti-inflammatory potential of diosgenin, quercetin, and crude fenugreek extract in reducing the production of pro-inflammatory cytokines (TNF-α and IL-6) and inhibiting NF-κB activation, which are key pathways in chronic inflammation. Diosgenin, a steroidal saponin, was found to be the most potent anti-inflammatory compound among those tested, followed by quercetin, a flavonoid, and crude fenugreek extract, which contains a mixture of bioactive compounds. These findings provide strong support for the therapeutic application of fenugreek, diosgenin, and quercetin as natural alternatives to conventional anti-inflammatory drugs, especially considering the well-documented side effects associated with long-term use of NSAIDs and corticosteroids. The results are particularly promising for individuals with chronic inflammatory conditions such as cardiovascular disease, diabetes, and neurodegenerative disorders, where inflammation plays a central role in disease progression.

The study also emphasizes the importance of investigating the individual contributions of bioactive compounds in fenugreek, as crude fenugreek extract exhibited moderate anti-inflammatory effects compared to diosgenin and quercetin in isolation. This suggests that while whole fenugreek extract may offer a range of benefits, the specific bioactive compounds such as diosgenin and quercetin are likely the primary contributors to its anti-inflammatory properties. This knowledge could inform the development of targeted nutraceuticals that leverage the individual benefits of these compounds.

In light of these findings, it is recommended that further research be conducted to explore the synergistic effects of diosgenin, quercetin, and other compounds found in fenugreek, such as alkaloids and saponins, to determine their combined efficacy in mitigating chronic inflammation. In addition to *in vitro* studies, *in vivo* studies should be prioritized to assess the systemic effects of these compounds and their potential for long-term use. Clinical trials in human populations are essential to determine the optimal dosages, bioavailability, and therapeutic windows for diosgenin and quercetin, as well as their ability to reduce inflammation in conditions such as rheumatoid arthritis, inflammatory bowel disease, and atherosclerosis. Additionally, the standardization of extraction methods and the identification of the bioactive compounds responsible for fenugreek's anti-inflammatory effects will be crucial in ensuring the reproducibility and clinical applicability of future studies.

On a practical level, the incorporation of fenugreek, diosgenin, and quercetin into functional foods or dietary supplements could provide a natural, safer alternative for managing chronic inflammation. Fenugreek seeds, due to

their rich phytochemical composition, could be incorporated into everyday diets in various forms, including powders, extracts, or as an ingredient in processed foods such as breads, smoothies, or soups. Additionally, diosgenin and quercetin could be formulated into standardized nutraceuticals to deliver targeted anti-inflammatory benefits, potentially improving patient adherence to treatment regimens due to their natural origin and fewer side effects. Furthermore, these findings highlight the need for awareness about the potential health benefits of functional foods among the general public. Public health initiatives could focus on promoting the use of plant-based anti-inflammatory foods as part of a holistic approach to preventing and managing inflammation-related diseases.

In conclusion, the study provides strong evidence for the anti-inflammatory effects of diosgenin, quercetin, and crude fenugreek extract, laying the foundation for the development of novel, plant-based therapies for chronic inflammatory conditions. By focusing on the isolation and standardization of fenugreek's bioactive compounds, and conducting further clinical research, the potential for fenugreek-based nutraceuticals to improve global health outcomes related to chronic inflammation is promising. Ultimately, fenugreek and its bioactive compounds could serve as key components in the shift toward more sustainable and natural therapeutic options in the management of chronic inflammatory diseases.

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