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Review Article

Determination Of *In-Vitro* Anti Inflammatory Activity of *Trigonella Foenum Graecum* and *Momordica Charantia* Linn

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ABSTRACT

The article reviews various in vitro assays that can be conducted in the laboratory to evaluate the anti-inflammatory activity of specific herbal extracts. Inflammation is a complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells, or irritants. It is typically characterized by redness, swelling, joint pain, stiffness, and loss of function. Although nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage inflammation, their long-term use is associated with serious side effects, including an increased risk of heart attacks and strokes. This has spurred interest in discovering safer, effective anti-inflammatory agents from natural sources. Medicinal plants, known for their rich chemical diversity, offer promising alternatives. Numerous phytoconstituents derived from herbal sources have demonstrated anti-inflammatory activity, although most findings remain at the experimental level with limited clinical validation. This review aims to compile and highlight data on key phytochemicals from medicinal plants that have shown potential in modern in vitro and in vivo inflammatory models.

INTRODUCTION

Inflammation is a normal and protective physiological response to tissue injury caused by physical trauma, harmful chemicals, or microbial agents. It plays a critical role in the body's defense mechanisms but can become problematic when dysregulated. There are two primary forms of inflammation:

1. **Acute Inflammation:** Characterized by increased vascular permeability, infiltration of capillaries, and migration of leukocytes to the site of injury.
2. **Chronic Inflammation:** Marked by persistent infiltration of mononuclear immune cells (such as macrophages, monocytes, and neutrophils), fibroblast activation, angiogenesis, and tissue fibrosis.

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Inflammation is a common underlying feature in many clinical conditions, including rheumatoid arthritis (RA)—a chronic, debilitating autoimmune disease[1] affecting approximately 1% of the population in developed nations[2]. The classical signs of inflammation include redness, swelling, heat, pain, and loss of function[3]. Among various mediators involved in inflammation, nitric oxide (NO) is a short-lived, gaseous free radical that plays a significant role. Modulation of NO synthesis or activity has been shown to alleviate both acute inflammation and experimental arthritis models[4,5]. NO is synthesized from L-arginine through the action of nitric oxide synthase (NOS) enzymes.

There are three major NOS isoforms:

- Two are constitutively expressed and calcium/calmodulin-dependent (referred to as cNOS).
- The third is the inducible isoform (iNOS), which is calcium-independent and upregulated in response to inflammatory cytokines[6].

Increased activity of nitric oxide synthase (NOS) and elevated nitric oxide (NO) levels are associated with both chronic and acute inflammation[7]. Notably, supplementing L-arginine, the amino acid precursor to NO, has been found to exacerbate inflammation, as evidenced by increased paw swelling in models of adjuvant-induced arthritis. To treat inflammatory conditions such as osteoarthritis, soft-tissue injuries, and bone fractures Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used[8]. Common NSAIDs include ibuprofen and naproxen. Another category of anti-inflammatory drugs includes glucocorticoids (e.g., cortisone, prednisone). While effective, these drugs are often associated with significant side effects such as gastrointestinal ulcers and bleeding, renal impairment, hypertension, hyperglycemia, and increased susceptibility to infections, particularly in patients treated with biological agents like tumor necrosis factor-alpha (TNF-α) blockers[9]. Moreover, the long-term use of synthetic anti-inflammatory drugs is limited by their toxicity and the recurrence of symptoms upon discontinuation. These limitations highlight the urgent need to explore safer and more effective alternatives. One promising avenue is the investigation of medicinal plants as sources of natural anti-inflammatory agents[10]. Unlike synthetic drugs that often target a single molecular pathway, herbal medicines typically contain a wide array of phytochemicals that work synergistically to modulate multiple pathways involved in inflammation[12]. Medicinal plants have historically served as a rich source of biologically active compounds and continue to play a crucial role in the treatment of various ailments. Their growing popularity is largely due to their perceived safety, reduced side effects, and holistic approach to healing[13]. India, possessing one of the largest repositories of medicinal plants in the world, holds a key position in the development and supply of herbal-based pharmaceuticals, cosmetics, and health supplements. With over 1.5 million practitioners of traditional medicine, the potential for harnessing indigenous plant resources for anti-inflammatory drug development is immense[14].

Fenugreek (*Trigonella foenum-graecum* L.)

Plant Profile:



(Fig 1)



(Fig 2)

Botanical Name: *Trigonella foenum-graecum* L.

Common Names:

- English : Fenugreek
- Hindi : Methi

Taxonomic Classification:

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae (Leguminosae)
Genus	Trigonella
Species	T. foenum-graecum

Origin and Distribution:

- The Mediterranean and Western Asia are believed to be the native regions of fenugreek.. It is widely cultivated in India, Egypt, Morocco, Turkey, France, and Argentina. India one of the largest producer and exporters of fenugreek seeds.
- **Habitat and Cultivation:**
- Fenugreek is a hardy annual plant suited to dry, semi-arid climates. It grows well in loamy or sandy soils with good drainage. It is typically sown as a winter crop in India and harvested between February and April.

Botanical Description:

- **Height**: 30–60 cm tall
- **Stem**: Erect, branched, and hollow
- **Leaves**: Compound trifoliate with oblong to obovate leaflets
- **Flowers**: Pale yellow to white, papilionaceous, usually solitary
- **Fruit**: Slender, curved pods containing 10–20 small hard seeds
- **Seeds**: Hard, yellow-brown, angular seeds with a characteristic bitter taste and pungent aroma

Growing Conditions:

a. Climate

- **Type**: Cool season, subtropical to temperate climates
- **Temperature**: Optimal growth occurs between **10°C to 30°C**
- **Frost Sensitivity**: Susceptible to frost, especially in the early seedling stage
- **Photoperiod**: Short-day plant but tolerates varied light durations **b. Soil**
- **Type**: Well-drained loamy to sandy-loam soils
- **pH Range**: Prefers slightly acidic to neutral pH (**6.0–7.0**)
- **Fertility**: Moderate; responds well to organic manures and nitrogen supplementation
- **Drainage**: Soil must have good drainage to prevent root rot

c. Watering

- Requires moderate watering
- Sensitive to waterlogging; irrigation should be reduced during maturity to avoid seed rotting
- Ideally irrigated 2–3 times depending on the soil and climate

d. Sowing and Harvesting

- **Sowing Time**:
- In India, Rabi season (October–November)

- Spring season in temperate regions (March–April)
- **Seed Rate**: 25–30 kg/ha
- **Spacing** : 30 cm between rows; 10 cm between plants

Harvesting:

- **Leaves** : 20–25 days after sowing (for fresh use)
- **Seeds**: 90–120 days after sowing, when the lower leaves start yellowing

Parts Used:

- **Seeds**: Most commonly used for culinary and medical purposes
- **Leaves**: Used fresh or dried (as "kasuri methi") in cooking
- **Whole plant**: Occasionally used in traditional medicine

Phytochemical Constituents:

- Fenugreek is rich in bioactive compounds, including:

Alkaloids	Trigonelline
Steroidal saponins	Diosgenin, yamogenin
Flavonoids	Quercetin, vitexin, isovitexin
Amino acids	4-Hydroxyisoleucine
Polysaccharides	Galactomannans (dietary fiber)
Phenolic acids	Caffeic, ferulic, gallic acids

- **Vitamins & Minerals**: Iron, magnesium, calcium, vitamin A, C, B-complex[16].

Traditional Uses:

- As an anti-inflammatory, antidiabetic, and digestive aid
- For inducing lactation in nursing mothers
- To reduce cholesterol and manage weight
- In treatment of menstrual cramps, indigestion, and wounds

Uses and Applications:

- Culinary: Leaves used as a leafy vegetable (methi); seeds as a spice
- Nutritional: Rich in fiber, protein, iron, saponins, flavonoids, and alkaloids
- Cosmetic and Pharmaceutical: Extracts used in skin care, hair growth products, and antiinflammatory formulations[17].

Pharmacological Activities:

a. Antidiabetic Effects of Fenugreek

Extensive preclinical and clinical evidence supports the hypoglycemic activity of fenugreek (*Trigonella foenum-graecum*). In vitro and animal studies demonstrate that fenugreek extracts enhance peripheral glucose uptake by increasing GLUT4 translocation and hexokinase activity, while simultaneously reducing hepatic gluconeogenesis through the downregulation of key enzymes such as glucose-6-phosphatase and fructose-1,6bisphosphatase. Additionally, fenugreek inhibits carbohydrate-digesting enzymes, including α-amylase and maltase, and exerts protective effects on pancreatic β-cells, thereby improving insulin secretion. Notably, the amino acid 4-hydroxyisoleucine isolated from fenugreek has been shown to stimulate insulin release from human and rat islets in a glucose-dependent manner. Other mechanisms include activation of AMP-activated protein kinase (AMPK) and modulation of incretin pathways, such as glucagon-like peptide-1 (GLP-1). Clinical trials consistently report that fenugreek seed supplementation improves glycemic control in patients with type 2 diabetes, significantly reducing fasting blood glucose, HbA1c levels, and enhancing insulin sensitivity. A recent review

confirms that multiple human studies have demonstrated fenugreek's efficacy in lowering blood glucose levels, improving insulin resistance, and beneficially altering lipid profiles. These antidiabetic effects are attributed to the synergistic actions of soluble fiber (galactomannan), which slows glucose absorption, and various bioactive compounds including trigonelline and 4-hydroxyisoleucine, which modulate insulin signaling pathways[18].

b. Hypolipidemic Effects of Fenugreek

Fenugreek (*Trigonella foenum-graecum*) has demonstrated significant lipidlowering (hypocholesterolemic) properties in both preclinical models and human clinical trials. Clinical studies have shown that daily consumption of germinated fenugreek seed powder (ranging from 25 to 100 g/day) leads to notable reductions in total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, and triglyceride (TG) levels, while preserving or even enhancing high-density lipoprotein (HDL) levels. In one clinical trial, hypercholesterolemic patients who received fenugreek supplementation experienced substantial decreases in TC and LDL cholesterol within one month. The hypolipidemic action of fenugreek is primarily attributed to its high content of soluble fiber and saponins, which bind bile acids in the intestine and promote their excretion, thereby compelling the liver to utilize circulating cholesterol for bile synthesis. Additionally, diosgenin, a steroidal saponin present in fenugreek, has been shown to inhibit intestinal cholesterol absorption. These mechanisms are further supported by meta-analyses of randomized controlled trials, with a recent systematic review of 15 studies reporting significant reductions in TC, TG, and LDL levels, along with a significant increase in HDL cholesterol. Collectively, these findings confirm that fenugreek exerts robust hypolipidemic effects, driven by its fiber content and bioactive steroidal compounds[19].

c. Anti-inflammatory and Antioxidant Activities of Fenugreek

Fenugreek (*Trigonella foenum-graecum*) exhibits significant anti-inflammatory and antioxidant properties, supported by a range of in vitro and in vivo studies. In vitro assays have demonstrated that fenugreek seed extracts effectively scavenge free radicals such as DPPH and ABTS, with hydroalcoholic extracts showing low IC_{50} values— indicative of strong radical-neutralizing potential. In lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cultures, fenugreek extract significantly inhibited the production of key pro-inflammatory mediators, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), prostaglandin E_2 (PGE_2), and nitric oxide, in a dose-dependent manner. In animal models of inflammation, such as carrageenan-induced peritonitis and air-pouch inflammation, fenugreek pre-treatment reduced leukocyte infiltration and oxidative tissue damage. These effects were accompanied by enhanced activity of endogenous antioxidant enzymes, including superoxide dismutase (SOD) and catalase, and reduced levels of oxidative stress markers such as malondialdehyde (MDA) and myeloperoxidase (MPO). These findings align with traditional medicinal uses of fenugreek for inflammatory disorders. The observed bioactivities are primarily attributed to its rich content of phenolic and flavonoid compounds, which are believed to modulate inflammatory pathways, including the nuclear factor-kappa B (NF-κB) signaling cascade, thereby attenuating inflammation and oxidative stress[20].

Medicinal Uses:

Use Case	Details / Mechanism	Evidence Type
Diabetes Management	Improves insulin sensitivity, promotes insulin secretion, reduces glucose absorption and gluconeogenesis	Clinical trials, in vitro animal studies
Cholesterol Reduction	Lowers total cholesterol, LDL, and triglycerides; fiber and saponins promote studies bile studies acid and excretion. Inhibits cytokines (e.g. TNF-α, IL-6),	Human and animal studies
Anti-inflammatory	suppresses COX-2/iNOS pathways, reduce oxidative stress	In vitro, animal model
Antioxidant	Scavenges free radicals (DPPH, ABTS), enhances SOD and catalase activity, lowers lipid peroxidation	In vitro, in vivo studies
Lactation Support (Galactagogue)	Stimulates milk production via phytoestrogenic activity.	Clinical reports, traditional use
Gastrointestinal Relief	Protects gastric lining, soothes indigestion, helps treat ulcers and acid reflux.	Animal models, traditional use
Hormonal Balance	May boost testosterone, manage menopausal symptoms via diosgenin and phytoestrogens.	Small clinical studies, traditional medicine
Antimicrobial	Inhibits growth of bacteria and fungi; shows potential in topical and digestive infections.	In vitro studies
Appetite Stimulation	Traditionally used to stimulate appetite in undernourished individuals.	Traditional medicine
Wound Healing	Enhances collagen synthesis, reduces inflammation, accelerates healing.	Preclinical studies
Weight Management	High fiber promotes satiety, reduces caloric intake.	Human and animal studies
Skin health	Used for treating acne, boils, eczema; anti-inflammatory and antimicrobial activity supports skin applications.	Traditional uses, preliminary studies.

Bitter Gourd (*Momordica charantia* L.)

Plant Profile:



(Fig 3)



(Fig 4)

Botanical Information:

- **Common Names** Bitter gourd, Bitter melon, Balsam pear, Karela (Hindi), Ampalaya (Filipino)
- **Botanical Name** *Momordica charantia* L.

- **Family** Cucurbitaceae
- **Plant Type** Climbing, tendril-bearing vine
- **Origin/Distribution** Native to tropical and subtropical regions of Asia, Africa, and the Caribbean

Botanical Description:

- **Habit**: Fast-growing vine with soft stems and climbing tendrils.
- **Leaves**: Deeply lobed (5–7 lobes), alternate, hairy, 4–12 cm wide.
- **Flowers**: Yellow, unisexual (separate male and female flowers), 2–4 cm in diameter.
- **Fruits**: Oblong or cylindrical, warty surface, green when unripe and orange when ripe; contains red arils enclosing seeds.
- **Seeds**: Flattened, brown or tan, encased in a fleshy coating[22].

Growing Conditions of Bitter Gourd:

a. Climate Requirements

- **Type**: Tropical and subtropical crop.
- **Temperature**: Optimal range is **24°C to 30°C**.
- **Frost Sensitivity**: Very sensitive to frost; should not be grown in cold or temperate climates during winter.
- **Sunlight**: Requires **full sun exposure** (minimum 6–8 hours of direct sunlight daily).
- **Rainfall**: Moderate rainfall (600–1200 mm annually) is suitable; avoid waterlogged conditions.

b. Soil Requirements

- **Soil Type**: Well-drained **loamy or sandy loam** soils are ideal.
- **Soil pH**: Slightly acidic to neutral (**pH 6.0 to 7.0**).
- **Fertility**: Rich in organic matter; compost or well-rotted manure is recommended before planting.
- **Drainage**: Good drainage is essential to prevent root rot.

c. Sowing and Propagation

- **Method**: Typically grown from **seeds**.
- **Seed Treatment**: Soaking seeds in water for 24 hours or treating with fungicide can improve germination.
- **Sowing Time**:
- **Tropical zones**: Two main seasons — *summer crop* (January–March) and *monsoon crop* (June–July).
- **Subtropical zones**: Sown after the last frost, typically in late spring.
- **Spacing**: 1.5–2 meters between rows; 0.6–1 meter between plants.

d. Irrigation

- **Frequency**: Regular, moderate watering; avoid overwatering.
- **Critical stages**: Germination, flowering, and fruit development.
- **Method**: Drip irrigation preferred for water efficiency and disease prevention[23,24].

Nutrient Management:

- **Basal dose**: Apply **farmyard manure (10–15 tons/ha)** before sowing.
- **Fertilizers**:
- **Nitrogen (N)**: 60–80 kg/ha
- **Phosphorus (P_2O_5)**: 40–60 kg/ha
- **Potassium (K_2O)**: 40–60 kg/ha
- Split application of nitrogen is recommended — half at sowing, half at flowering[25].

Trellising and Support:

- **Trellis system**: Recommended for high yield and better-quality fruits.
- **Height**: 1.5–2 meters; allows fruits to hang freely and reduces pest/disease issues.

Pest and Disease Management:

a. Common Pests:

- Fruit fly
- Red pumpkin beetle
- Aphids

b. Common Diseases:

- Downy mildew
- Powdery mildew
- Mosaic virus

c. Control Measures: Integrated Pest Management (IPM), neem oil sprays, crop rotation, and resistant varieties.

d. Harvesting

- **First Harvest**: 55–70 days after sowing, depending on the variety.
- **Harvest Stage**: Fruits are harvested **when young and green**, before seeds harden.
- **Harvest Frequency**: Every 2–3 days during peak season to encourage new fruiting. **e. Yield**
- **Average Yield**: 10–15 tons/ha under normal conditions; can reach up to 20 tons/ha with improved practices[26].

Phytochemical Constituents:

- **Group** : **Examples**
- **Triterpenoids :** Charantin (noted for hypoglycemic activity)
- **Polypeptides** : Polypeptide-p (plant insulin)
- **Alkaloids :** Momordicine
- **Flavonoids** : Quercetin, kaempferol
- **Glycosides** : Cucurbitacin B
- **Saponins** : Momordicosides
- **Vitamins & Minerals** : Vitamin C, A, B-complex, iron, calcium, potassium

Parts Used:

- Primarily **fruit** (unripe and ripe), but also **leaves**, **seeds**, and **roots** in some practices.

Traditional Uses:

- Used in Ayurveda and Traditional Chinese Medicine for diabetes, skin diseases, liver disorders, and digestive complaints.
- In folk medicine, bitter gourd juice is commonly consumed to "purify blood" and manage blood sugar.
- Leaf paste applied topically for skin infections and wounds[27].

Medicinal Uses:

Antidiabetic Activity

- **Primary Use**: Most well-known for its **blood glucose-lowering effects**.
- **Mechanism**: Enhances insulin secretion, improves glucose uptake, inhibits gluconeogenesis, and contains insulin-like compounds such as polypeptide-p and charantin.
- **Evidence**: Supported by numerous clinical studies and animal models.

Antioxidant Properties

- **Effect**: Bitter gourd scavenges free radicals and reduces oxidative stress.
- **Active Compounds**: Flavonoids, vitamin C, phenolic acids.

- **Benefit**: Protects cells from damage, slows aging, and may help prevent chronic diseases.

Anticancer Effects

- **Action**: Induces apoptosis (programmed cell death) in cancer cells, inhibits tumor growth and proliferation.
- **Evidence**: Shown effective in lab studies against **breast, prostate, liver, and colon cancers**.
- **Mechanism**: Modulates signaling pathways and oxidative stress.

Hepatoprotective (Liver Protection)

- **Benefit**: Protects the liver from toxins and promotes liver function.
- **Uses**: Traditionally used for **jaundice**, **fatty liver**, and liver detox.
- **Mechanism**: Antioxidant activity and modulation of liver enzymes[28]. Anti-inflammatory and Immunomodulatory
- **Effect**: Reduces inflammation by inhibiting pro-inflammatory cytokines (like TNF-α, IL)
- **Immune Support**: Enhances macrophage and lymphocyte activity.

Antimicrobial Activity

- **Spectrum**: Effective against various **bacteria, fungi**, and some **viruses**.
- **Traditional Uses**: Used to treat infections, wounds, and skin diseases.

Digestive Aid

- **Function**: Stimulates digestion, helps relieve **constipation**, **flatulence**, and **indigestion**.
- **Bitterness**: Increases secretion of digestive enzymes and bile.

Skin Health

- **Traditional Use**: Treats **eczema**, **psoriasis**, **acne**, and **ringworm**.
- **Topical application**: Leaf paste or juice applied to affected skin areas.

Antiviral Effects

- **Evidence**: Some extracts inhibit replication of viruses including **HIV**, **herpes**, and **hepatitis B** in lab settings[29].

Anti-obesity and Lipid-lowering Effects

- **Action**: Lowers triglycerides and LDL cholesterol, helps regulate fat metabolism.
- **Weight Management**: Suppresses appetite and fat accumulation in animal studies. **Menstrual Regulation**
- **Traditional Use**: Used in herbal formulations to regulate **irregular menstruation** and cleanse the uterus.

Note: Caution in pregnancy due to potential uterine stimulant effects.

Wound Healing

- **Application**: Fresh juice or paste promotes healing of **cuts**, **sores**, and **insect bites**.
- **Effect**: Antimicrobial and anti-inflammatory action accelerates tissue repair.

Key Bioactive Compounds:

- **Charantin** – Hypoglycemic compound
- **Polypeptide-p** – Insulin-like protein
- **Momordicin** – Antiviral and anti-inflammatory
- **Cucurbitacins** – Antitumor activity
- **Vitamins C, A** – Antioxidants[30].

CONCLUSION:

In-vitro anti-inflammatory assays offer a efficient and animal-friendly approach to evaluate herbal extracts. In-vivo studies requires more time when compare to in-vitro methods and fewer animal resources. Herbal drugs tend to have fewer side effects than synthetic NSAIDs. Various invitro assays, such as protein denaturation, membrane lysis, and enzyme inhibition, can be conducted in a laboratory setting, utilizing techniques like spectrophotometry, and in some cases, human blood samples. This approach enables straightforward assessment of herbal anti-inflammatory properties.

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