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Tulsi (Ocimum Sanctum): Immunomodulatory and Antimicrobial Activity

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Abstract : *Infectious diseases and immune dysregulation remain major health problems, and the rise of antimicrobial resistance has renewed interest in medicinal plants. Tulsi (holy basil), Ocimum sanctum Linn. (synonym Ocimum tenuiflorum; family Lamiaceae), is a sacred plant of India that has been used for thousands of years in Ayurveda for respiratory, febrile, digestive and skin disorders. The leaves contain a volatile oil rich in eugenol, together with caryophyllene, methyl eugenol and other terpenes, and phenolic compounds and flavonoids such as rosmarinic acid, apigenin and luteolin, as well as ursolic acid. Experimental studies report anti-inflammatory, analgesic, antipyretic, antioxidant, immunomodulatory, antimicrobial, antistress and gastroprotective activities of leaf extracts and seed oil. Cyclooxygenase inhibition by eugenol and phenolic compounds, modulation of humoral and cellular immune responses and antibacterial activity against enteric pathogens have been described. A double-blind placebo-controlled trial in healthy volunteers reported changes in immune parameters after leaf extract, and a systematic review of human studies concluded that the evidence is promising but limited by small and heterogeneous trials. This article reviews the history, phytochemistry, experimental protocols, mechanisms, benefits, applications, advantages and limitations of Tulsi with emphasis on immunomodulatory and antimicrobial activity.*

Keywords: TIAA :- Tulsi, Ocimum sanctum, Immunomodulatory, Antimicrobial, Eugenol

I. INTRODUCTION

The immune system protects the body against infection and tumours, and its dysregulation is involved in infections, allergies, autoimmune disease and cancer [1]. Antibiotic resistance is a growing problem worldwide, and the side effects of immunosuppressive and anti-inflammatory drugs limit their use. Plants with a long history of safe use are therefore attractive sources of new therapeutic agents [2,3].

Ocimum sanctum Linn., known as Tulsi or holy basil, is an aromatic herb that is cultivated in Indian homes and temples and is regarded as sacred [4]. It is called the “Queen of Herbs” and “The Incomparable One” in Ayurveda, and it is used for coughs, colds, fever, bronchitis, malaria, skin disease and digestive complaints [5,6]. The essential oil is rich in eugenol, which has been studied for anti-inflammatory and analgesic actions [7].

Animal studies have shown that Tulsi leaf extract and seed oil have anti-inflammatory, analgesic and antipyretic effects [8,9], that its phenolic compounds inhibit cyclooxygenase enzymes [10], and that the seed oil modulates immune responses [11]. A randomised trial in healthy volunteers reported changes in immune parameters [12], and a systematic review of human trials has summarised the available clinical evidence [13]. This article presents a structured review of Tulsi as an immunomodulatory and antimicrobial plant.

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II. LITERATURE SURVEY

1. Godhwani, S. et al. (1987)
Alcoholic extract of Ocimum sanctum leaves showed anti-inflammatory activity in carrageenan-induced paw oedema in rats and analgesic and antipyretic activities in animal models, supporting its traditional use. [8]

2. Maulik, G. et al. (1997)
Several Indian herbal plants, including Ocimum sanctum, were evaluated for antioxidant effectiveness, and the leaf extract showed free-radical scavenging activity. [14]

3. Singh, S. & Majumdar, D. K. (1997)
Fixed oil of Ocimum sanctum seeds, rich in unsaturated fatty acids, showed anti-inflammatory activity in rat models, and the effect was linked to inhibition of arachidonic acid metabolism. [9]

4. Kelm, M. A. et al. (2000)
Phenolic compounds isolated from Ocimum sanctum, including eugenol and rosmarinic acid, showed antioxidant and cyclooxygenase inhibitory activity in vitro. [10]

5. Geeta Vasudevan, D. M. et al. (2001)
Ocimum sanctum extract showed activity in vitro against a number of enteric pathogens, supporting its traditional use in gastrointestinal infections. [15]

6. Mediratta, P. K. et al. (2002)
Ocimum sanctum seed oil enhanced humoral and cell-mediated immune responses in rodents and the effect was suggested to involve modulation of immune pathways. [11]

7. Gupta, S. K. et al. (2002)
This study validated several traditional claims of Tulsi in animal models, including anti-stress, anti-inflammatory and antiulcer effects. [16]

8. Prakash, P. & Gupta, N. (2005)
This review summarised the pharmacological actions of Ocimum sanctum and eugenol, including antimicrobial, anti-inflammatory, analgesic, antidiabetic and immunomodulatory effects. [7]

9. Mondal, S. et al. (2011)
In a double-blind, placebo-controlled trial in healthy volunteers, Tulsi leaf extract altered immune parameters, including T-helper cell and natural killer cell counts and interferon-gamma levels. [12]

10. Jamshidi, N. & Cohen, M. M. (2017)
This systematic review of human studies concluded that Tulsi may benefit metabolic, immune and stress-related conditions, with mild side effects, but that the trials were small and of variable quality. [13]

Historical Background of Tulsi (Ocimum sanctum):
Tulsi has been revered in India for thousands of years. It is mentioned in the ancient Vedic literature and in the Ayurvedic texts, and it holds a religious position in Hindu households, where it is planted in a special pot and worshipped daily [4,5]. Traditional systems of medicine such as Ayurveda, Siddha and Unani recommend Tulsi for cough, cold, fever, bronchitis, malaria, diarrhoea, skin disease, snake bite and as a general tonic [6,17].
The plant spread to other tropical regions and is cultivated in Southeast Asia, Africa and the Middle East. Modern research started in the middle of the twentieth century, and systematic pharmacological work on anti-inflammatory, analgesic, antipyretic and antistress activity was published from the 1980s [8,16]. More recently, immunomodulatory and clinical studies have been published [12,13], and Tulsi tea and supplements are now sold worldwide.
PROTOCOL :

III. MATERIALS

1. Plant Material
Fresh leaves of Ocimum sanctum are collected from cultivated plants or herbal gardens and identified by a qualified botanist [18]. The leaves are washed with water, shade-dried at room temperature for several days to preserve volatile

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constituents, powdered and stored in airtight containers. A voucher specimen is deposited in the institutional herbarium. For essential oil, fresh leaves are used.

2. Chemicals and Reagents

Analytical-grade ethanol, methanol, petroleum ether and distilled water are used for extraction [19]. Nutrient agar, Mueller–Hinton agar and standard antibiotic discs are used for antimicrobial assays [20]. Sheep red blood cells are used as antigen in immunological studies, and carbon ink is used for the carbon clearance test [21,22]. Carrageenan is used for inflammation studies [23], and Folin–Ciocalteu reagent and DPPH are used for antioxidant assays [24,25]. Levamisole or cyclophosphamide may be used as reference immunostimulant or immunosuppressant.

3. Experimental Animals

Healthy Wistar rats (150–200 g) or Swiss albino mice (20–25 g) of either sex are acclimatised for one week under standard laboratory conditions with free access to pellet diet and water. Experiments are approved by the Institutional Animal Ethics Committee and follow national regulations and the ARRIVE guidelines [26].

4. Extraction Equipment and Methods

Leaf powder is extracted by Soxhlet extraction or maceration with aqueous, ethanolic or petroleum ether solvents. Essential oil is obtained by hydrodistillation in a Clevenger apparatus, and seed oil is obtained by solvent or cold-press extraction. Extracts are concentrated under reduced pressure and stored at 4 °C.

5. Analytical Instruments

GC-MS is used to identify volatile constituents such as eugenol, caryophyllene and methyl eugenol, and HPLC is used for rosmarinic acid, ursolic acid and flavonoids [7,10]. A UV–visible spectrophotometer, a plethysmometer and a haemagglutination setup are used for the respective assays. A microplate reader is used for MTT and microdilution assays.

6. Phytochemical Screening

Qualitative tests are carried out to detect alkaloids, flavonoids, tannins, saponins, terpenoids, phenolic compounds and glycosides [18,19]. Total phenolic and flavonoid contents are estimated by the Folin–Ciocalteu and aluminium chloride methods [24,27].

IV. METHODS

1. Preparation of Tulsi Extracts

Typically 50 g of powdered leaf is extracted successively with petroleum ether, ethanol and water in a Soxhlet apparatus for 6–8 hours per solvent, concentrated and dried. The percentage yield is calculated.

2. Phytochemical Analysis

Qualitative tests, total phenolic content and total flavonoid content are determined for each extract, and the major constituents are identified by GC-MS and HPLC [24,27].

3. In Vitro Antimicrobial Assays

Agar Well or Disc Diffusion: Standardised bacterial or fungal suspensions are spread on agar plates and the zone of inhibition around wells containing extract is measured and compared with standard antibiotics [20]. Broth Microdilution: The minimum inhibitory concentration (MIC) is determined as the lowest concentration of extract that prevents visible growth, and the minimum bactericidal concentration (MBC) is determined by sub-culture.

4. In Vitro Antioxidant and Anti-inflammatory Assays

DPPH radical scavenging, lipid peroxidation inhibition and inhibition of albumin denaturation are measured spectrophotometrically [25,28,29]. Cyclooxygenase inhibition can be measured with commercial enzyme kits [10].

5. In Vivo Immunomodulatory Studies

Humoral Immunity: Animals are immunised with sheep red blood cells and the antibody titre is measured by haemagglutination [21]. Cell-mediated Immunity: Delayed-type hypersensitivity is measured as footpad thickness after antigen challenge. Phagocytic Function: The carbon clearance test is used to measure the phagocytic index [22].

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Treatment with extract is given orally for several days, and results are compared with a control group and a standard drug.

6. In Vivo Anti-inflammatory and Analgesic Studies

Carrageenan-induced paw oedema is measured with a plethysmometer after carrageenan injection [23]. Analgesic activity is measured by the hot-plate or acetic acid-induced writhing test [30,31].

6. Toxicity Studies

Acute oral toxicity of Tulsi preparations is evaluated in rodents following OECD Guideline 423 (acute toxic class method), in which a single oral dose, commonly up to 2000 mg/kg, is administered and the animals are observed for 14 days for mortality, behavioural changes and body-weight alterations [32]. Sub-acute toxicity is evaluated by repeated daily dosing for 28 days according to OECD Guideline 407, followed by estimation of haematological and serum biochemical parameters and histopathology of vital organs [33].

7. Histopathological Analysis

At the end of an experiment the spleen and thymus is excised, fixed in 10% neutral buffered formalin, dehydrated through graded alcohols, cleared, embedded in paraffin and sectioned at about 5 µm. Sections are stained with haematoxylin and eosin (H&E) and examined under a light microscope for inflammatory cell infiltration, tissue architecture and degenerative changes by a pathologist who is blinded to the treatment groups [30].

8. Statistical Analysis

In the experimental studies summarised in this review, data are generally expressed as mean ± SEM. Group comparisons are performed by one-way analysis of variance (ANOVA) followed by a post hoc test such as Tukey's or Dunnett's test, and $p < 0.05$ is usually taken as the threshold of statistical significance. Reporting of animal experiments is expected to follow the ARRIVE guidelines [26].

V. MECHANISM OF ACTION

Tulsi acts through several routes. Its volatile oil and phenolic compounds influence inflammatory enzymes, immune cell function and microbial growth [5,7].

1. Immunomodulation:

Tulsi seed oil enhanced antibody production and cell-mediated immune responses in animals [11], and in healthy volunteers leaf extract increased the number of helper T cells and natural killer cells and the level of interferon-gamma [12]. These observations indicate stimulation of both humoral and cellular immunity.

2. Cyclooxygenase Inhibition and Anti-inflammatory Action:

Eugenol and phenolic compounds from Ocimum sanctum inhibit cyclooxygenase enzymes and thereby reduce prostaglandin synthesis [10,34]. The fatty acids of the seed oil inhibit arachidonic acid metabolism through both cyclooxygenase and lipoxygenase pathways [9].

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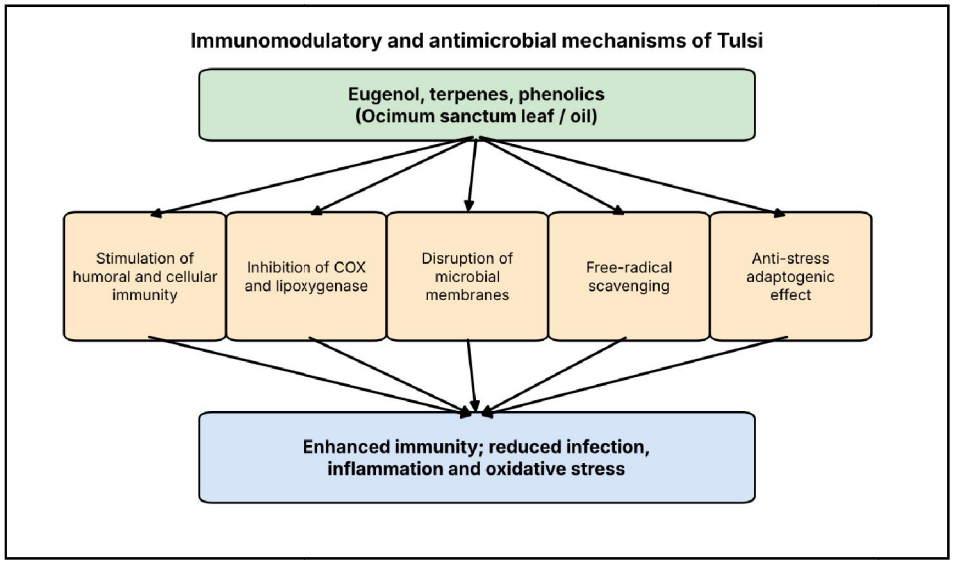


Fig.No.1. Mechanism of Action of Tulsi

3. Antimicrobial Action:

Eugenol and other terpenoids disrupt microbial cell membranes and interfere with enzyme function, which results in growth inhibition. Tulsi extracts have shown in vitro activity against enteric bacteria [7,15].

4. Antioxidant Activity:

Phenolic compounds and flavonoids scavenge free radicals and inhibit lipid peroxidation [10,14,35].

5. Antistress Action:

In animal studies, Tulsi protected against stress-induced changes in ulcer index and adrenal parameters, which is in keeping with an adaptogenic effect [5,16].

6. Possible Anticancer Mechanisms:

Experimental studies suggest that Tulsi constituents induce apoptosis, protect against carcinogen-induced damage and modulate detoxifying enzymes, but clinical evidence is lacking [36].

VI. OVERALL MECHANISM SUMMARY:

The immunomodulatory and antimicrobial actions of Tulsi result from the combined effect of eugenol and other terpenes and phenolic compounds, which stimulate immune responses, inhibit cyclooxygenase and lipoxygenase pathways, scavenge free radicals and inhibit microbial growth.

Health Benefits of Tulsi (Ocimum sanctum)

Tulsi is used as a general tonic, and many specific health benefits have been described [4,6].

1. Immunomodulatory Activity

Experimental and human studies suggest enhancement of humoral and cellular immune responses [11,12].

2. Antimicrobial Activity

Tulsi extracts and oil inhibit bacteria and fungi in vitro, including enteric pathogens [7,15].

3. Anti-inflammatory, Analgesic and Antipyretic Activity

Leaf extract and seed oil reduce inflammation, pain and fever in animal models [8,9].

4. Antioxidant Properties

Phenolic compounds scavenge free radicals [10,14].

5. Respiratory and Digestive Health

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Tulsi is used traditionally for cough, cold, bronchitis and digestive complaints, and animal studies show antiulcer activity [6,16].

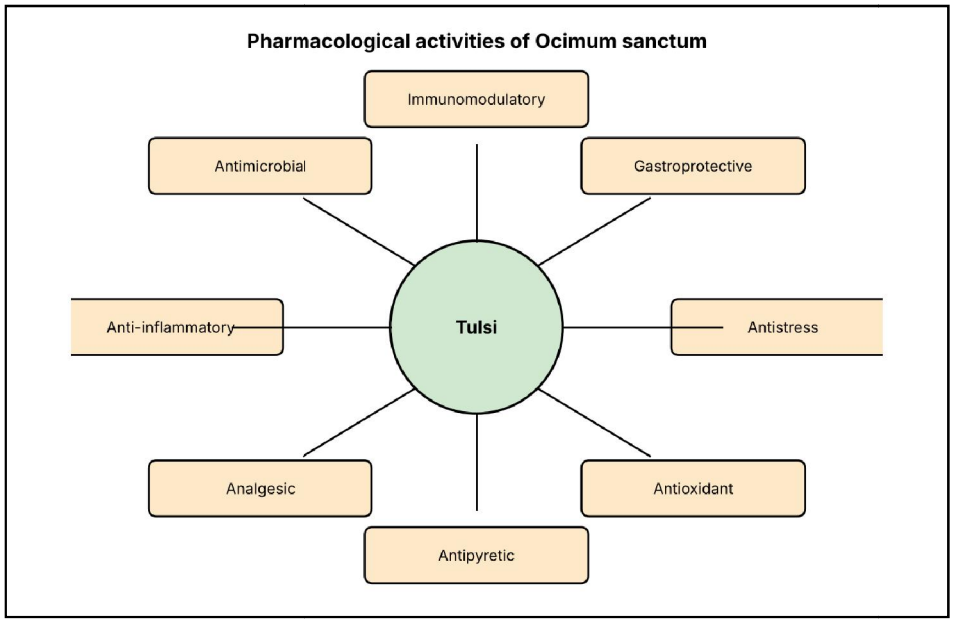


Fig.No.2. Pharmacological Activities of Tulsi

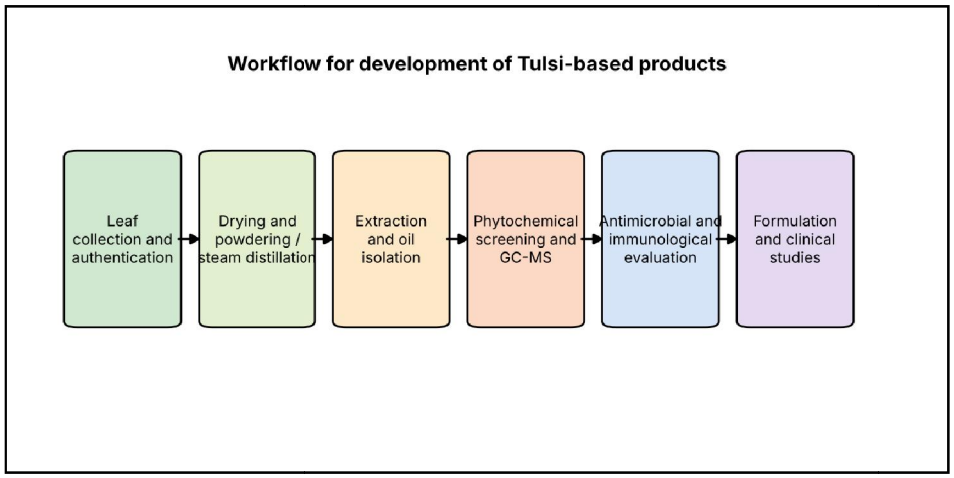


Fig.No.3. From Tulsi Leaf to Formulation and Evaluation

6. Antistress and Cognitive Support

Animal studies and some human trials suggest an anti-stress effect [13,16].

7. Metabolic Effects

Systematic review data suggest possible benefits on blood glucose and lipids, but the trials are small [13].

8. Anticancer Potential

Preclinical studies suggest chemopreventive effects [36].

Reported Findings from Experimental Studies

1. Phytochemical Analysis

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Leaf extracts contain eugenol, caryophyllene, methyl eugenol, rosmarinic acid, apigenin, luteolin, ursolic acid, tannins and saponins, and the composition varies with variety, season and extraction method [7,17].

2. In Vitro Antimicrobial Activity

Extracts and oil inhibit the growth of several bacteria and fungi in disc and well diffusion assays, with the effect depending on the solvent and organism [15,17].

3. In Vivo Immunomodulatory Studies

Seed oil and leaf extract increased antibody titre and delayed-type hypersensitivity in animals [5,11].

4. Anti-inflammatory and Analgesic Studies

Leaf extract and seed oil reduced carrageenan-induced paw oedema, and analgesic and antipyretic effects were seen in rodents [8,9].

5. Clinical Studies

A systematic review of human studies reports favourable effects on metabolic, immune and stress-related outcomes, with mild adverse effects such as nausea, but it also notes small sample sizes and poor trial quality [13].

6. Toxicity Studies

Acute toxicity in animals is low, and in human trials adverse events were mild [13,16]. High doses of eugenol can cause irritation and, at very high doses, toxicity.

Applications of Tulsi (Ocimum sanctum)

Tulsi has medicinal, culinary, cosmetic and agricultural applications.

1. Medicinal Applications

Immune Support: Leaf extract and tea are used to support immunity [12].

Antimicrobial: Used for respiratory and gastrointestinal infections traditionally [6].

Anti-inflammatory and Analgesic: Used for fever, pain and inflammation [8].

Antistress: Used as an adaptogen [16].

2. Culinary and Beverage Applications

Tulsi leaves are used in herbal teas and as a flavouring [4].

3. Cosmetic and Oral Care Applications

Tulsi extracts and eugenol are used in toothpastes, mouthwashes, skin creams and hand sanitisers because of their antimicrobial and fragrance properties [7].

4. Agricultural and Environmental Applications

Tulsi oil and extracts have been tested as natural insect repellents and in stored grain protection [17].

5. Traditional and Folk Medicine

The leaf juice is taken with honey for cough and cold, and the leaf paste is applied to skin disease and insect bites [5,6].

Advantage and limitations of Tulsi :

Advantages of Tulsi

1. Very long history of traditional use [4].
2. Easily cultivated and available.
3. Immunomodulatory activity in animals and humans [11,12].
4. Antimicrobial activity including against enteric pathogens [15].
5. Anti-inflammatory, analgesic and antipyretic activity [8].
6. Antioxidant activity [14].
7. Adaptogenic effect [16].
8. Mild adverse effects in human trials [13].
9. Safe for use as tea and food ingredient.
10. Multiple uses in medicine, cosmetics and agriculture [7,17].

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VII. LIMITATIONS OF TULSI

1. Human trials are small and of variable quality [13].
2. Composition varies with variety, season and extraction [17].
3. Optimum dose and duration not established [13].
4. In vitro antimicrobial activity may not translate to clinical effect.
5. Possible effect on blood glucose and clotting needs caution with antidiabetic and anticoagulant drugs [13].
6. High doses of eugenol are irritant.
7. Long-term safety data are lacking.
8. Use in pregnancy is not recommended as safety is not established.
9. Quality control of commercial products is a concern [37].
10. Many studies are in animals [5].

VIII. FUTURE PROSPECTS OF TULSI:

1. Pharmaceutical Development
- Development of standardised leaf extracts and isolated constituents.
- Evaluation of eugenol and ursolic acid derivatives [7].

2. Clinical Trials
- Large randomised trials on immune function and infection.
- Standard protocols and validated endpoints [13].

3. Antimicrobial Research
- Activity against drug-resistant organisms.
- Synergy with conventional antibiotics.

4. Immunology Research
- Detailed study of effects on cytokines and immune cell subsets [12].
- Use in vaccination and convalescence.

5. Nutraceutical Applications
- Herbal teas and functional foods.
- Standardised supplements.

6. Drug Delivery Systems
- Nanoemulsions of essential oil for improved stability.
- Topical and oral controlled-release formulations.

7. Combination Therapies
- Combination with other herbs and conventional drugs.
- Reduction of antibiotic dose.

8. Oral and Dental Care
- Mouthwashes and gels with Tulsi extract.
- Clinical trials in gingivitis.

9. Cosmetic Industry
- Antimicrobial skin-care products.
- Natural fragrance and preservative.

10. Agricultural Applications
- Natural insecticides and repellents.
- Post-harvest protection.

11. Food Industry
- Natural preservative and flavour.
- Antimicrobial packaging.

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12. Anti-cancer Research
- Mechanism studies on chemoprevention [36].
- Combination with standard therapy.

13. Metabolic Disorder Management
- Trials in diabetes and dyslipidaemia [13].
- Functional supplements.

14. Quality Control and Certification
- Chemical fingerprints of varieties.
- Standards for global trade.

15. Integration with Modern Medicine
- Evidence-based integration with conventional care.
- Public education on safe use.

IX. SUMMARY

Tulsi (Ocimum sanctum) is a sacred and medicinal plant of India with a long history of use for infections, fever, cough and as a general tonic. It contains eugenol, other terpenes, phenolic compounds and flavonoids. In vitro studies show antimicrobial and antioxidant activity, and in vivo studies show immunomodulatory, anti-inflammatory, analgesic, antipyretic and antistress effects. Mechanisms include cyclooxygenase and lipoxygenase inhibition, stimulation of immune responses and microbial membrane disruption. A randomised trial in healthy volunteers reported altered immune parameters, and a systematic review suggests benefits with mild side effects but poor trial quality. The main limitations are small clinical trials, variable composition, unclear dose and limited long-term data. Future work should focus on standardisation and rigorous clinical studies [13,17].

X. CONCLUSION

Tulsi shows promising immunomodulatory and antimicrobial activity in experimental studies and encouraging early human data. With standardised preparations and well-designed clinical trials, Tulsi may become a useful complementary agent for immune support and for the management of infections and stress-related conditions.

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