

Phytochemical analysis, characterization, antioxidant, anticariogenic and biocompatibility of *Ocimum sanctum*

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Abstract

Introduction: *Ocimum sanctum* (Tulsi), a herb with a long history in Ayurvedic medicine, is known for its broad therapeutic benefits. It contains bioactive compounds like terpenoids, flavonoids, and phenolic compounds, which have antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties. Tulsi's extracts are used to treat conditions like bronchitis, rheumatism, epilepsy, skin diseases, and digestive disorders. Modern studies highlight its wound-healing effects by promoting tissue regeneration and reducing inflammation. Tulsi's antimicrobial action, especially against antibiotic-resistant bacteria, offers potential as a natural alternative to conventional antibiotics. It also supports digestive health by enhancing bile secretion and enzyme activity. Overall, Tulsi is a promising natural remedy that warrants further research for its medicinal applications. **Materials and Methods:** Tulsi (*Ocimum sanctum*) extract is prepared by washing, drying, and grinding the leaves, followed by methanol extraction and solvent partitioning. Phytochemical screening identifies the presence of compounds like alkaloids, flavonoids, tannins, saponins, and terpenoids through various chemical tests. The antioxidant activity is assessed using the DPPH radical method, and anti-inflammatory effects are measured by testing against Bovine Serum Albumin. Antimicrobial activity is determined using the agar well diffusion method, and anti-biofilm activity is evaluated using a microtiter plate assay. Biocompatibility is tested through an MTT assay using L929 fibroblast cells to assess cytotoxicity and cell viability. This comprehensive approach helps confirm the therapeutic potential of Tulsi.

Results: The test substance shows a dose-dependent inhibition, starting at 17.72% at 10 µg/ml and increasing steadily to 88.50% at 50 µg/ml. This rising inhibition with higher doses suggests strong anti-inflammatory properties, as the substance effectively prevents protein denaturation at increasing concentrations. The plot for DPPH assay showed a dose-dependent increase in radical scavenging activity (%RSA) of the test substance, starting at 24% at 20 µg/ml and reaching nearly 60% at 100 µg/ml. This linear increase demonstrates a strong correlation between higher doses and enhanced antioxidant activity, typical of compounds with potent free radical scavenging properties. The test substance shows a dose-dependent reduction in biofilm formation, with slight inhibition at 25 µg/ml and more pronounced effects at 50 µg/ml and 75 µg/ml. At 100 µg/ml, biofilm formation is significantly reduced across bacterial species, indicated by much lighter staining. This suggests the compound effectively inhibits biofilm development, particularly at higher concentrations. **Discussion:** *Ocimum sanctum* (Tulsi) exhibits potent antioxidant, anti-inflammatory, antibacterial, and anticariogenic properties due to its high content of flavonoids, phenolics, and essential oils. Its antioxidant action helps protect against oxidative stress, which is linked to diseases like cancer and cardiovascular issues. The plant also shows broad-spectrum antimicrobial effects, inhibiting pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus mutans*, while reducing biofilm formation and dental plaque. Biocompatibility studies suggest that *O. sanctum* is safe for use in medical, cosmetic, and pharmaceutical applications. Further research is needed to explore its full therapeutic potential. **Conclusion:** Tulsi, or *O. sanctum*, possesses antioxidant, anticariogenic, and anti-inflammatory properties, making it a promising therapeutic agent for various illnesses. However, further research is needed to address safety, bioavailability, and clinical applications.

Keywords: *Ocimum sanctum*, phytochemical analysis, antioxidant, anticariogenic, biocompatibility, herbal medicine.

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Introduction

Natural products are rare and valuable that contain safer, highly efficient antimicrobial agents. New advances in analytical science and technology, especially in phytotherapy, resulted in an innovative age of herbal products as well as anti-plaque treatments (1). *Ocimum sanctum* (Tulsi), a well-known culinary and medicinal fragrant herb native to the Indian subcontinent and belonging to the Lamiaceae family, has been used in Ayurvedic medicine for more than three thousand years (2). Tulsi is known as a "Elixir of Life" for its healing abilities and has been used to treat a number of conditions. Tulsi leaf extracts are used to treat bronchitis, rheumatism, and pyrexia. Treatment of epilepsy, asthma or dyspnea, hiccups, cough, skin and hematological illnesses, parasite infections, neuralgia, headache, wounds, and inflammation, and oral problems are among the other known medicinal uses (3). Pharmacological and clinical studies showed that *O. sanctum* consists of terpenoids, iridoids, phenolic compounds, and flavonoids. These active components act as antimicrobial, anti-oxidative, anti-inflammatory, anti-arthritic, and antipyretic agents. Only a few studies use *O. sanctum* as an anti-oxidative agent (4). Most of the phytochemical constituents of *O. sanctum* alter the microbial organism's structural and functional integrity (5). Beyond its traditional medical history, *O. sanctum* has significant medical applications. The plant's medicinal properties are starting to be supported by modern studies, especially with regard to how well it treats wounds. *O. Sanctum*'s wound-healing effects are facilitated by bioactive substances, including flavonoids and essential oils, which promote tissue regeneration, enhance collagen synthesis, and reduce inflammation at the site of damage. This is particularly important in clinical settings where preventing infections and promoting a quicker recovery depend on efficient wound treatment(6). *O. sanctum* may also be useful in the fight against infections resistant to antibiotics due to its strong antibacterial qualities. The global increase in antibiotic resistance has heightened the hunt for substitute antimicrobial medicines. According to studies, *O. sanctum* extracts can stop the growth of a variety of bacteria and fungi, including those that cause gastrointestinal problems and skin infections(7). The herb's promise as a natural substitute or addition to

conventional antibiotics is highlighted by its broad-spectrum antibacterial action, particularly when it comes to treating infections that are challenging to treat with current pharmaceutical treatments(8). Finally, it is impossible to ignore the advantages of *O. sanctum* for digestive health. Both traditional use and early scientific research provide strong evidence for its capacity to reduce gastrointestinal distress and promote the digestive process. The herb may help treat digestive disorders like dyspepsia and irritable bowel syndrome because of its ability to increase bile secretion and enhance the activity of digestive enzymes(9). *O. sanctum* provides a comprehensive approach to health care that is in line with the expanding trend of incorporating natural medicines into modern medicine by promoting gut health and enhancing digestive function. When taken as a whole, these elements highlight the significance of *O. sanctum* in medicine and the necessity of further study into its possible treatments.

Materials and Methods

Tulsi extract preparation

The leaves were washed with sterile water and shade-dried for 1 week and ground coarsely and extracted with methanol at room temperature. The extracts were combined and concentrated in vacuum at 30 °C. The residue was diluted with water and partitioned against petroleum ether, ethyl acetate and n-butanol and each were concentrated to dryness to obtain powder. Phytochemical screening of *Ocimum sanctum* (commonly known as Tulsi) from its powdered form involves a series of procedures to identify the presence of different classes of compounds like alkaloids, flavonoids, tannins, phenolic acids, saponins, and terpenoids.

Procedure:

1. Preparation of Plant Extract: Weigh about 50 g of powdered *Ocimum sanctum*. Soxhlet extraction using ethanol as a solvent was performed. Allow the solvent to circulate for several cycles to thoroughly extract the compounds. Filter the solution using filter paper, and evaporate the solvent using a rotary evaporator to obtain a concentrated extract. The extract can be redissolved in a small amount of solvent to carry out different qualitative tests for phytochemicals.

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2. Phytochemical Screening Tests: The following tests can be performed on the plant extract to detect the presence of different classes of compounds:

a. Alkaloids Detection (Wagner's Test): To 2-3 mL of the extract, add a few drops of Wagner's reagent (iodine in potassium iodide).

Observation: A reddish-brown precipitate indicates the presence of alkaloids.

b. Flavonoids Detection (Shinoda Test): To 2-3 mL of the extract, add magnesium turnings and a few drops of concentrated hydrochloric acid (HCl).

Observation: A pink or red color after a few minutes indicates the presence of flavonoids.

c. Tannins Detection (Ferric Chloride Test): Add a few drops of 0.1% ferric chloride solution to 2-3 mL of the extract.

Observation: A blue-black or greenish color indicates the presence of tannins.

d. Phenolic Acids Detection (Ferric Chloride Test): Add 1% ferric chloride solution to a small amount of extract.

Observation: A dark green or blue coloration indicates phenolic acids.

e. Saponins Detection (Froth Test): Mix the extract with water and shake it vigorously. Observation: A stable, persistent froth indicates the presence of saponins.

f. Terpenoids Detection (Salkowski Test): Mix 2 mL of extract with 2 mL of chloroform. Add 2 mL of concentrated sulfuric acid (H₂SO₄) carefully along the side of the test tube.

Observation: A reddish-brown interface indicates the presence of terpenoids.

g. Glycosides Detection (Keller-Kiliani Test): To 2 mL of the extract, add glacial acetic acid, a few drops of ferric chloride solution, and concentrated sulfuric acid. Observation: A brown ring at the interface indicates the presence of glycosides.

h. Essential Oils Detection (Steam Distillation): For essential oils, perform steam distillation of the powdered plant material and collect the distillate. The distillate can be analyzed using GC-MS (Gas Chromatography-Mass Spectrometry) for detailed profiling of individual essential oil components.

3. Documentation: the color changes, precipitates, or frothing results from each test were recorded carefully. Each test result will help to confirm the presence or absence of specific phytochemicals.

DPPH assay - Antioxidant activity The antioxidant activity of extract and Ascorbic acid was measured in

terms of electron transfer/hydrogen donating ability, using the DPPH radical method of modified by the extract at various concentrations of 20,40,60,80,100 µg/ml was added to 3.9 ml of DPPH radical solution. The decrease in absorbance at 515 nm was determined continuously at every 1 min with a UV-Visible Spectrophotometer:

Anti inflammatory activity

The fraction was dried in a vacuum oven and re-dissolved in iso saline. Different concentrations of the fractions were made and added to 1.8 ml of 1 % Bovine Serum Albumin solution. The pH was adjusted to 6.5 using 1N HCl and the solution was incubated at 37 °C for 20 min and then heated to 57 °C for 10 min. After cooling, the absorbance was measured at 660 nm. Aspirin was used as the standard and a solution without sample/extract was considered as the control.

Anti Microbial activity

The agar well diffusion method was used to determine the antibacterial activity of *Ocimum sanctum*. Different concentrations of 25µg, 50µg, 75µg and 100µg were incorporated into the wells and the plates were incubated at 37°C for 24 hrs. The antibiotics like streptomycin and vancomycin were used as positive control. Zone of inhibition was recorded in each plate after 24 hours.

Anti biofilm activity

The anti-biofilm activity of a test compound was evaluated against *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Shigella sonnei* using a 96-well microtiter plate assay at concentrations of 25 µg, 50 µg, 75 µg, and 100 µg, with untreated biofilms serving as the control. Bacteria were initially cultured in vinyl U-bottom 96-well plates, where they were allowed to form biofilms over a 24-48 hour incubation period at optimal growth conditions, such as 37°C. After incubation, the planktonic (free-floating) bacterial cells were carefully washed away using a gentle rinsing buffer like phosphate-buffered saline (PBS), ensuring that only the biofilm adhered to the well surface remained. To visualize the biofilm, the wells were stained using crystal violet dye, which binds specifically to the biofilm's extracellular polymeric substance (EPS) matrix, providing a visual representation of biofilm density. Once stained, the excess dye was removed, and the wells were rinsed again to remove unbound crystal violet. For quantification, the stained biofilms were solubilized using ethanol or an equivalent solvent to dissolve the crystal violet. The solubilized biofilms were then transferred to a flat-bottom, optically clear 96-well plate

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to allow for accurate spectrophotometric measurement at a wavelength of 570 nm. This method enabled the quantification of biofilm mass based on absorbance readings. The results were compared between the treated groups at different concentrations (25 µg, 50 µg, 75 µg, and 100 µg) and the untreated control group. A reduction in absorbance values in the treated wells, compared to the control, would indicate the compound's effectiveness in inhibiting biofilm formation, with higher concentrations potentially demonstrating greater anti-biofilm activity. This assay provides a quantitative means of evaluating how well a compound can disrupt or prevent biofilm formation by these clinically relevant bacterial species.

Biocompatibility tests

The biocompatibility of the material was evaluated through an MTT assay, a colorimetric method used to measure cell metabolic activity, which serves as an indicator of cell viability and proliferation. Cell viability was assessed after a 24-hour incubation period, during which cells were exposed to the test material. The L929 mouse fibroblast cell line, commonly used in biocompatibility and cytotoxicity studies, was selected for this assay. These cells are known for their sensitivity to foreign materials, making them ideal for assessing the compatibility of the test material. L929 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with fetal bovine serum (FBS) and necessary growth factors to ensure optimal cell health. The cells were maintained at standard culture conditions (37°C, 5% CO₂) to simulate physiological environments. After 24 hours of exposure to the test material, the MTT reagent was added to the culture wells. MTT is a tetrazolium salt that is reduced to formazan crystals by the mitochondrial enzymes of metabolically active cells. The formazan product was then solubilized, and absorbance readings were taken at a wavelength of 570 nm using a microplate reader. The absorbance values directly correlate with the metabolic activity of the cells, which reflects their overall viability. Higher absorbance indicates greater metabolic activity and cell health, suggesting that the material is non-cytotoxic, while lower absorbance would indicate potential cytotoxic effects. This detailed process allows for accurate determination of the biocompatibility of the material based on how it impacts the viability of L929 mouse

Results

1. Flavonoids: A class of plant secondary metabolites with antioxidant properties.

The specific flavonoids listed are:

- Apigenin
- Luteolin
- Quercetin
- Rutin

2. Phenolic Acids: Compounds with potential anti-inflammatory and antioxidant activities:

- Caffeic Acid
- Chlorogenic Acid
- Salicylic Acid

3. Essential Oils: Aromatic compounds extracted from plants, often used for therapeutic purposes. The essential oils listed include:

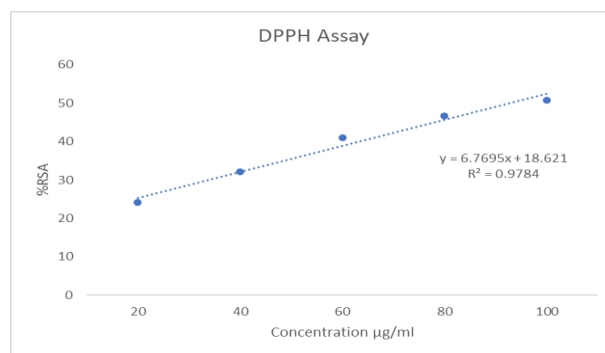
- β-Pinene
- Sabinene
- Camphor
- α-Terpineol

4. Tannins: Polyphenolic compounds known for their astringent properties and antioxidant potential:

- Catechins

5. Terpenoid: A large class of organic compounds produced by plants, often contributing to scent and flavor. Terpenoids listed include:

- α- and β-Selinene
- Germacrene-D
- Bornyl Acetate
- Borneol • Sabinol

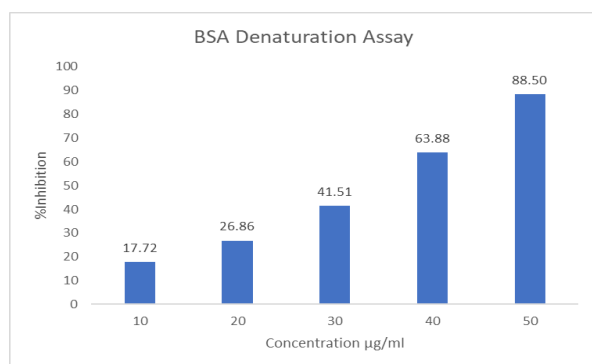


a) AntiOxidant Assay : DPPH Assay Antioxidant Activity:

DPPH Assay is a common method to evaluate the free radical scavenging ability of a compound, which correlates with its antioxidant potential.

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- The plot shows a dose-dependent increase in the percentage of radical scavenging activity (%RSA) with increasing concentrations of the test substance (in $\mu\text{g/ml}$).
- At the lower end of the concentration spectrum (around 20 $\mu\text{g/ml}$), the %RSA is approximately 24%.
- As the concentration rises to 100 $\mu\text{g/ml}$, the %RSA increases steadily, reaching nearly 60%.
- This linear increase, described by the equation, reflects a strong dose-dependent response, meaning the antioxidant activity is proportional to the amount of the test substance administered.
- The value suggests a very high correlation, indicating that increasing the dose of the test compound consistently enhances the free radical scavenging activity. This is a typical observation in compounds with potent antioxidant properties, where higher doses neutralize more radicals



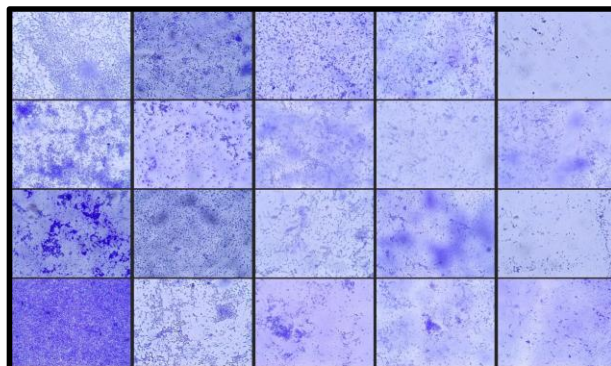
b) Anti-inflammatory assay

Bovine Serum Albumin Denaturation Assay (BSA)

Anti-inflammatory Activity :

- The BSA Denaturation Assay assesses the ability of a substance to inhibit protein denaturation, which is an important process in inflammation. Inflammation often leads to the denaturation of proteins, and inhibiting this process suggests anti-inflammatory potential.
- The bar graph presents a clear dose-dependent inhibition of protein denaturation with increasing concentrations of the sample (from 10 to 50 $\mu\text{g/ml}$).
- At 10 $\mu\text{g/ml}$, the test substance shows about 17.72% inhibition.
- As the dose increases, the inhibition steadily rises:
 - 26.86% at 20 $\mu\text{g/ml}$,
 - 41.51% at 30 $\mu\text{g/ml}$,
 - 63.88% at 40 $\mu\text{g/ml}$,
- Peaking at 88.50% inhibition at the highest concentration of 50 $\mu\text{g/ml}$.

- This dose-dependent increase in inhibition suggests that the test substance's ability to prevent BSA denaturation (and hence, inflammation) becomes significantly stronger as the dose increases. Such a pattern is often observed with anti-inflammatory agents, where higher doses exhibit more pronounced effects on inflammatory processes.



c) Anti Biofilm activity against *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Shigella sonnei* at 25 μg , 50 μg , 75 μg and 100 μg vs Control: untreated

I. Assay Setup:

- Bacteria Tested:
 - *Enterococcus faecalis*
 - *Staphylococcus aureus*
 - *Streptococcus mutans*
 - *Shigella sonnei*
- Concentrations of the Test Compound:
 - 25 $\mu\text{g/ml}$
 - 50 $\mu\text{g/ml}$
 - 75 $\mu\text{g/ml}$
 - 100 $\mu\text{g/ml}$
- Control Group: Untreated, where no test compound was used to evaluate the natural formation of biofilms without interference.

II. Staining and Biofilm Visualization:

- The biofilms are stained to make them visible, with darker staining (deeper blue/purple) indicating thicker or more mature biofilm formation. Lighter staining represents reduced biofilm formation.

III. Interpretation of the Images:

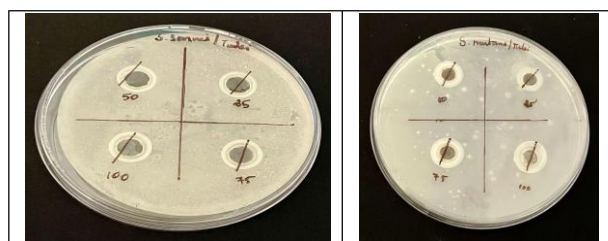
- Top Row (Control): These images show the bacterial biofilm formation without any treatment. You can observe thick, dense biofilms with darker purple/blue staining, especially for some bacterial species. This

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demonstrates the natural capacity of these bacteria to form biofilms when untreated.

Rows with Treatment (25 µg/ml to 100 µg/ml): As the concentration of the test substance increases, a dose-dependent reduction in biofilm formation can be observed. The biofilm becomes progressively lighter in color, indicating reduced bacterial adherence and matrix formation. At 25 µg/ml: There is a slight reduction in biofilm formation compared to the untreated control, but biofilms are still present. At 50 µg/ml and 75 µg/ml: The reduction in biofilm formation is more pronounced. The bacteria are less able to form dense biofilms, and the staining becomes lighter. At 100 µg/ml: The biofilm formation is drastically reduced in most bacterial species, indicated by the much lighter staining, suggesting that the test compound has significantly inhibited biofilm development at this highest concentration.

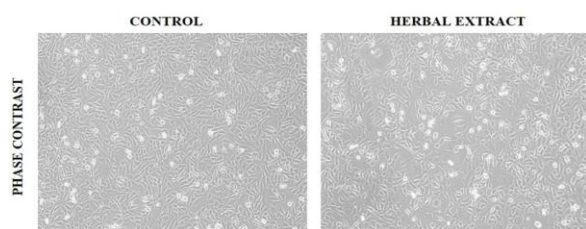
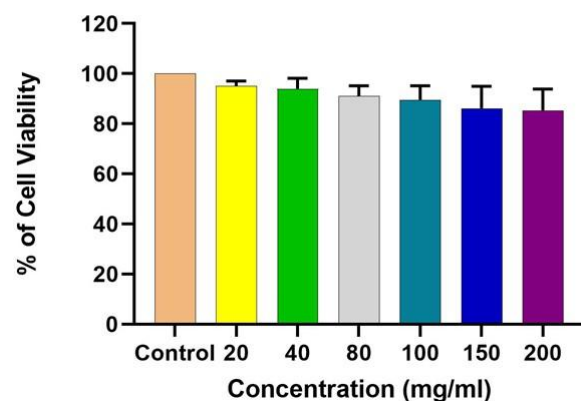
	Zone of inhibition	
Samples µg/ml	Streptococcus mutans (+)	Shigella sonnei (-)
25	13mm±0.1	13mm±0.1
50	14mm±0.1	13mm±0.1
75	15mm±0.1	14mm±0.1
100	16mm±0.2	15mm±0.1



d) Concentration-Dependent Activity: Both dishes show that higher concentrations of the Tulsi extract (75 and 100 µg/mL) produce larger zones of inhibition, indicating stronger antibacterial activity. Comparative Efficacy: The table allows for comparison between the effects of the Tulsi extract on both types of bacteria. For instance, at 100 µg/mL, the inhibition zones for *S. mutans* are slightly larger than those for *S. sonnei*, suggesting that

Tulsi might be slightly more effective against *S. mutans* under these conditions.

MTT ASSAY



e) MTT assay results and the phase contrast microscopy images collectively suggest that the Tulsi extract is biocompatible with L929 fibroblast cells. The extract does not significantly reduce cell viability even at higher concentrations. The Tulsi treated cells retain their normal morphology, similar to the control cells. This indicates that Tulsi extract is non-toxic to these cells and could be considered safe for further studies or applications related to fibroblast health and tissue repair.

Discussion

Antioxidant Activity: Research continuously demonstrates *O. sanctum*'s strong ability to scavenge free radicals, which considerably lowers oxidative stress. This antioxidant ability is essential for protecting cells against oxidative damage, which is a major factor in the etiology of long-term illnesses such as cancer, neurological diseases, and cardiovascular disease. (10) Studies further reveal that Tulsi's high phenolic and flavonoid content is responsible for some of its robust antioxidant properties, indicating that it may be used as a treatment for illnesses associated with oxidative stress. *O. sanctum* has a rich mix of beneficial chemicals, according to phytochemical research. Flavonoids, alkaloids, tannins, sesquiterpene lactones, and essential oils are a few of these. The plant's

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therapeutic qualities are mostly due to the presence of these chemicals. (5)

The pharmacological effects of flavonoids like luteolin and apigenin, as well as essential oils that contain camphor and chamazulene, are especially noteworthy.

The high concentration of flavonoids and phenolic chemicals in *O. sanctum* is thought to be responsible for its antioxidant action. By neutralizing free radicals, these substances can stop oxidative stress and the resulting cellular damage. *O. sanctum* extracts have been demonstrated in studies to have strong free radical scavenging activity, suggesting that it may be a natural source of antioxidants(11). *O. sanctum*'s flavonoids and sesquiterpene lactones are primarily responsible for its noteworthy anti-inflammatory qualities. These substances prevent the synthesis of pro-inflammatory cytokines and enzymes, like COX-2, which are essential in the inflammatory reaction. Because of its anti-inflammatory properties, it can be used to treat illnesses and situations that cause inflammation(12). *O. sanctum*'s anticariogenic properties are especially significant when it comes to oral wellness. The plant's antibacterial qualities aid in preventing the development of cariogenic organisms like *Streptococcus mutans*. Furthermore, the tannins' presence adds to the decrease in tooth decay and dental plaque development, making it an important natural agent in dental attention. Broad-spectrum antibacterial activity of *O. sanctum* is demonstrated against a range of pathogens, such as viruses, fungi, and bacteria. This action is mostly brought about by phenolic chemicals and essential oils(13). Research has indicated that the plant's extracts can effectively combat common bacteria like *Staphylococcus aureus* and *Escherichia coli*, as well as fungi like *Candida albicans*.

A vital consideration for *O. sanctum* applications in medicine and therapy is biocompatibility. Studies show that at therapeutic doses, plant extracts are generally harmless and do not harm human cells. It is therefore a strong contender for usage in a range of pharmaceutical and medical products. *O. sanctum* has a great deal of potential for medicinal uses due to its varied phytochemical composition and variety of bioactivities(14). Its biocompatibility and antibacterial, anti-inflammatory, anticariogenic, and antioxidant qualities make it an important natural resource in contemporary medicine. It is necessary to do additional research and clinical investigations to fully understand its potential and create efficient formulations for a range of medical ailments. Research has shown that the bioactive

components of *O. sanctum*, such as flavonoids and sesquiterpene lactones, efficiently prevent the growth of a variety of bacteria, including *Escherichia coli* and *Staphylococcus aureus*(15). Moreover, *O. sanctum* inhibits the formation of biofilms, which is essential for avoiding infections and enhancing dental health. Studies reveal that *O. sanctum* extracts lessen oral bacteria's acid production, which plays a role in dental caries development. Research indicates that the chemicals derived from *O. sanctum* exhibit minimal cytotoxicity and favorable biocompatibility with human cells, implying their safety for application in herbal remedies, cosmetic products, and medicines. *O. sanctum* is therefore a viable option for long-term therapeutic use(16). Further bolstering *O. sanctum*'s safety profile are *in vitro* investigations that demonstrate it does not cause appreciable harm in human fibroblasts and epithelial cells.

Limitations

The consistency and repeatability of therapeutic effects may be impacted by phytochemical variability. Variations in the duration of harvesting and techniques used for processing (such as extraction and drying) might affect the concentration and potency of bioactive chemicals. While difficult, standardizing these processes is essential to guaranteeing constant quality. It takes efficient delivery methods to improve solubility and stability. Robust clinical trials to confirm safety in humans are scarcer. The active ingredients in Tulsi, especially the flavonoids and phenolic acids, have a low bioavailability, which can restrict their potency. Sophisticated methods for thorough phytochemical profiling, as well as long-term clinical trials evaluating the tolerability and safety, will offer vital information for its safe application. Its economic success and wider adoption can be fueled by identifying and expanding niche markets, such as supplements for anti-inflammatory conditions or dental care.

Conclusion

Tulsi, or *O. sanctum*, has potent antioxidant qualities that fend off free radicals, shielding cells and enhancing general health. Its anticariogenic properties prevent the growth of cariogenic bacteria, improving dental health by lowering cavities and plaque. Additionally, it has anti-inflammatory qualities, which increases the health advantages and possible uses of it. The herb is a safe and flexible substance because of its biocompatibility, which

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guarantees safe contact with living tissues. *O. sanctum* is a highly promising therapeutic agent; nonetheless, its incorporation into contemporary healthcare will depend on resolving existing restrictions and advancing research into its safety, bioavailability, and clinical uses. Overcoming these obstacles will enable Tulsi to be used to treat a wide range of illnesses naturally and efficiently.

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