

# THERAPEUTIC POTENTIAL OF TULSI-LOADED NANOGELS IN COMBATING BACTERIAL AND FUNGAL INFECTIONS

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**Abstract:** The objective and the goal of the research study is to develop a new herbal gel formulation on the treatment of fungal infection by incorporating a powder extract of Tulsi (*Ocimum sanctum* leaves). Topical gel formulations are applied in the skin. A fungal infection is one of the most important problems in the health care system of the world and can present itself in various forms, posing a threat of chronic diseases and disorders in the case of insufficient treatment. Gels are semi-solid mixtures that are basically liquid mixtures that are thickened using other products. Another well used Ayurvedic medicine is Tulsi (*Ocimum sanctum* linn) which has antimicrobial effects that are commonly used to treat several systemic illnesses. Sacred basil (*Ocimum sanctum*) includes active components like alkaloids, enzymes and other inorganic components in all its properties.

**Keywords:** *Ocimum sanctum* Linn leaves extract, antibacterial, antifungal, *E. coli*, *Aspergillus Niger*, Eugenol.

## **Introduction:**

The term nanogel was originally used to describe small, cross-linked polymer networks that comprise of a charged polymer (polyion) and a neutral polymer. This was attributed to the fact that these networks were able to carry genetic materials like polynucleotide (1).

Nogel have gained great attention because they can be used to deliver drugs in the targeted, controlled delivery and long delivery (2).

Nanogels are nanosized nano-sized hydrogel of hydrogel that have their diameter in the nanometres range (3).

Their size Characterised by size larger surface area and hygroscopicity Range The size is typically between 20 and 200 nanometres. They may be incorporated in transdermal delivery system, and these are nontoxic and no irritating and thus increases the bioavailability and solubility of the drug (4).

Tulsi (holy basil) belongs to the family Lamiaceae with the botanical name of *Ocimum sanctum*. Tulsi is also known as the Queen of Herbs because of its numerous medicinal values besides large use in the herbal medicine. There are two simple types of Tulsi that have close similar properties, but these are; “Green Tulsi (Ram Tulsi) and black Tulsi (Krishna Tulsi). The tradition has one of its major symbols tulsi (holy basil) with a very religious meaning in the Hindu religion (5).

Tulsi has been rated to be one of the highest medicinal plants in Ayurveda and the symbol of complete

health and well-being. Tulsi is bitter in flavor which is characteristic (6).

Tulsi is a plant practiced in Ayurveda which has thousands of curative properties since thousands of years. The plant is useful in all the parts including leaves and seeds. Tulsi has been identified as a source of natural energy and was also a source of physical power and immunity booster (7).



**Fig 1 : Ocimum sanctum (Tulsi)**



**Fig 2 : Leaves of Ocimum sanctum**

**Geographical source:**

Holy basil is available in most countries, including India, Burma, srilanka, Bangladesh, China, Southwest Asia, thiland, furthermore, tulsi is also present in dry sand areas like Cambodia, Indonesia, laos, myanmar etc<sup>(8)</sup>.

**Taxonomical classification of tulsi kingdom**

Kingdom	Plantae
Sub kingdom	Tracheobionta
Superdivision	Spermatopta
Division	Magnoliophyte
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae
Genus	Ocimum
Species	O. sanctum

**Morphology :**

<b>Distrubution</b>	Grow upto 30-60cm height <sup>(9)</sup>
<b>Territory</b>	Found through the Indis <sup>(9)</sup>
<b>habit</b>	Annual herb <sup>(9)</sup>
<b>Mool</b>	Thiny, wiry, branched hairs, soft, colour black to brown from external or dull and violet internal <sup>(9)</sup>

<b>Tana</b>	Stiff, greenly, wooded, branches are hairy, external colour pinkish-brown to black, internal colour dull yellowish, fracture: stringy and slightly aromatic odour <sup>(9)</sup>
<b>Patra</b>	Leaves are 2.5 to 5 cm long, 1.6 to 3.2 cm wide, elliptically egg-shaped, with a dumb or acute apex, hairy on both sides. Petiole is thin, hairy; odour-aromatic <sup>(9)</sup>
<b>Chaal</b>	Colour: greyish brown outside and pale pink from inside, longitudinal cracks are present. <sup>(9)</sup>
<b>Pushpa</b>	Purplish or crimson coloured, calyx elliptical or campanulated 3-4mm bilipped, odour is aromatic, taste is pungent. <sup>(9)</sup>
<b>Phal</b>	Four nutlets, each contains one seed membranous, colour is dull brown reddish with small black patches, odour is aromatic and taste is pungent. <sup>(9)</sup>
<b>Beej</b>	Shape is oval, colour is brown, mucilaginous when soaked in water, odourless, taste is pungent <sup>(9)</sup>

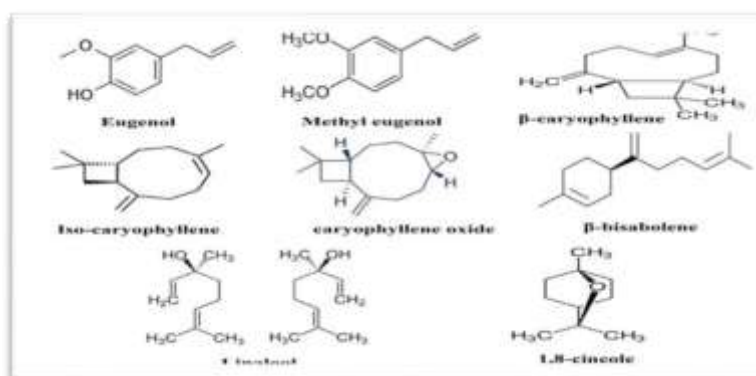
### Botanical Description:

Its leaves smell good and they are basic and laid in opposite pairs. They are oval or somewhat long and reach a length of approximately 5 cm.

Tulsi is a plant whose flowers are small and purple to reddish in colour. They are small and are cylindrical spikes. The inside of the outer part of the flower is smooth, and the tube of the flower contains minute hairs. The flowers take an average length of less than 5 mm. Tulsi bears little fruits, and the seed is yellow to reddish in colour (10)

### Phytochemistry of *Ocimum sanctum*:

The chemical structure of Tulsi is complicated and includes a great number of nutrients and active natural compounds (11). *Ocimum sanctum* has approximately 0.7 volv. oil in its leaf. The oil is mostly composed of eugenol and methyl eugenol 71-percent and 20-percent, respectively. It also contains other substances as carvacron and caryophyllene among others. Moreover, flavonoids including orientin and vicenin are present in the tulsi leaf water extract(12).



**Critical components of essential oil:** The critical active components of the essential oil are Eugenol (or methyl eugenol) and methyl chavicol. The remaining one present in essential oil are 2-caryophyllene, 2-caryophyllene oxide and germacrene D (13).

**Phenyl propanoids Eugenol:** Eugenol is extremely important in pharmaceutical business and is customarily isolated out of the Tulsi leaves. The most common phenylpropanoid in the essential oil of the Leaves of *Ocimum sanctum* is Eugenol. There are also others that are related compounds that can be found in the leaves like ociglycoside (synonymously referred to as eugenyl-B-D-glucoside). Ferulaldehyde citrussin C and dehydrodieugenol. These plant leaves are also extracted ones of *Ocimum sanctum* plant (15).

**Neolignans:** Neolignans are a type of compounds that is found in the methanolic extract of the OS. they are formed through the combination of the eugenol molecules within the cell (15).

**Flavonoids:** Flavonoids are the major active plants of the plant, *Ocimum sanctum*. These are the methoxy flavonoids and the glycosides of the latter such as cirsimartin, isothymusin as well as luteolin. The c-glycosides flavonoids available in the plant are vicenin, isovitexin, isoorientin and orientin. (16) Other flavones were also identified by APCI-MS. These are cirsumaritin, crisilineol, isothymusin, gardenin, apigenin, eupatorin and salvigenin. (17)

**Fatty acid derivatives:** These are cerebrosides which are fatty acid derivatives that occur in the root and leaves of the OS plant. The OS leaves have palmityl glucoside and sanctumoic acid that makes it affect its killing of mosquitoes (18).

**Fixed oil:** Fixed oil is also known as non-volatile oil extracted with the help of the seeds of the OS plant which contain approximately 18.22 percent of the fixed oil. It contains some health benefits, including anti-inflammatory, blood pressure reduction, prevention of cancer, and the thinning of the blood, the primary component of this oil is the linoleic acid that makes up 66.1 percent of it (19).

**Other fatty acids** other than linoleic acid are 2.1 stearic acid, 9.0 oleic acid, 6.94 palmitic acid, and 15.7 alpha-linolenic acid. Interestingly these are also positive compounds that are present in the plant leaves of the OS plant (20).

**Terpenoid:** *Ocimum sanctum* (tulsi) is a plant, which has a great number of important natural materials known as terpenoids. They are classified into different types namely: sesquiterpenoids, diterpenoids and triterpenoid, some of the major compounds found in the plant are: beta-caryophyllene, carnosic acid, ursolic acid, oleanolic acid, and beta-amyrin- glucopyranoside (21).

**ursolic acid** is the most abundant in the plant. Scientists confirmed this using lab techniques including HPTLC and UPLC-ESI-MS/MS (22).

**Other terpenoids tulsi** contains include elements, humulene, germa-cres, bergamotene and hydroxy caryophyllene all of which contribute to the medicinal qualities of the plant (23)

**Coumarins:** three coumarins are isolated out of the tulsi plant which is aculetin, aesculin and ocimamarin (24).

**Steroids:** 3-O- 2 -D-glucopyranoside: Beta-sitosterol, beta-sitosterol-3-O-2-D- glucopyranoside, stigmasterol, and campesterol are steroids that are found in *Ocimum sanctum* (Tulsi). It is through these steroid compounds derived using stem and leaves of the plant. (25)

**Antibacterial and Antifungal activity:**

almost all species of *Ocimum* produce essential oils that exhibit significant antifungal, antibacterial and antiviral activity. Infections caused by antibiotic-resistant bacteria have become a major therapeutic challenge, and medicinal plants are considered a promising alternative to overcome this problem. Tulsi leaves have been reported to possess strong antifungal activity against *Aspergillus* species. In addition, the essential oil of *Ocimum gratissimum* L. has demonstrate invitro antifungal activity against *Aspergillus* species by disrupting fungal cell membrane, causing leakage of cellular contents and inhibiting growth<sup>(26)</sup>.

**Method of preparation of Nanogel: Emulsion solvent diffusion method:**

The aqueous solution of the drug is solubilised in an organic phase. The polymer and gelling agent are dissolved in water to prepare drug phase. The drug phase is then added dropwise to the aqueous phase and homogenised for 30 minutes at 6000rpm. During homogenization, the emulsion is converted into nanodroplets using a homogeniser, resulting in the formulation of an oil-in-water (o/w) emulsion.<sup>(27)</sup>

Triethanolamine is added to the oil in water emulsion to form a nanogel and the mixture is continuously stirred at 8000rpm per hour.<sup>(28)</sup>

**Nano precipitation method :**

When the organic phase containing both the drug and polymer was extracted with the aqueous surfactant phase, the polymer precipitated. After removing the excess solvent polymeric nanoparticle remained.<sup>(29)</sup>

The gelling agent and the required amount of nanoparticle dispersion are added after the particles have been properly moistened. The pH is then stabilised using triethanolamine.<sup>(30)</sup>

**Evaporation of the solvent method :**

During the hours of treatment the drug polymer mixture is injected into the the designed region of the aqueous phase. The process is carried out under continuous stirring at 1000rpm using a magnetic stirrer.<sup>(31)</sup>

The nanosponges obtained as consequence are further subjected to filtration, followed by a drying process in a hot air oven maintained at a temperature of 40 C 24 hours<sup>(32)</sup>

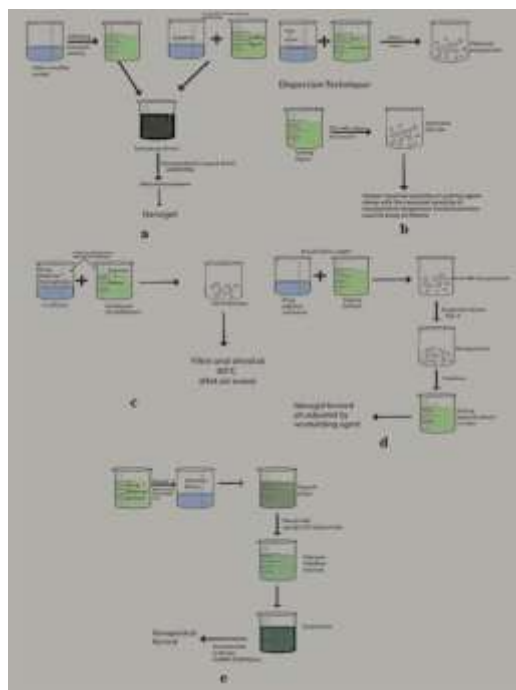
Finally, the dried nanosponges are carefully transported into vials for storage. To achieve homogeneous dispersion, it is recommended to immerse the polymer in water for 2 hours before the initiation of gel formation.<sup>(33,34)</sup>

The polymer was subjected to agitation at a rotational speed of 6000 rpm. the ph is modified with the use of pH adjusting agent. Subsequently, aqueous dispersion is combined with the optimised nanospoges suspension and permeation enhancer.<sup>(35,36)</sup>

**Modified Diffusion emulsification:**

A polymer dissolved in a suitable solvent is mixed with a drug in a precisely calculated ratio. The organic phase is formed by continuously agitating the drug polymer mixture in the aqueous phase at a speed 5000-10000rpm.<sup>(37)</sup>

a syringe fitted with a needle is used to add the organic phase at a rate of 0.5ml per minute to the aqueous stabiliser solution after being agitated for 6 minutes at rpm 10000-25000 rpm. The suspension was subjected to sonication for 5-10minuts.<sup>(38)</sup>



**Fig 4:method of preparation nanogel Evaluation:**

- 1) Spreadability test.
- 2) Physical Appearance (colour, odour)
- 3) Stability testing.
- 4) PH measurement.
- 5) Clarity
- 6) Viscosity

## Conclusion:

The article sheds light on therapeutic potential of Tulsi (*Ocimum sanctum*) leaf extract loaded nanogels in terms of bacterial and fungal infection. A substance, tulsi, that has always been considered a potent Ayurvedic medicine with antimicrobial, anti-inflammatory and immunomodulatory effects, is a substance with a broad spectrum of bioactive compounds (eugenol, flavonoids, terpenoids, fatty acid derivatives) that contribute to its broad-spectrum action. The experiment becomes a new method of enhancing the solubility stability and bioavailability of drugs, in addition to controlled and targeted delivery by incorporation of Tulsi extract in nanogel formulations.

Nanogels provide a better delivery system as a transdermal system in comparison with conventional gels due to their high surface area, nanoscale and biocompatibility. The preparation techniques, namely: emulsion solvent diffusion, nanoprecipitation and modified diffusion emulsification, make it possible to create stable formulation with good physicochemical properties. The parameters of such assessment as the spreadability, the viscosity, the pH and the stability evidence the possibility of the use of these nanogels as topical agents.

Generally, Tulsi-loaded nanogels represent a fresh, greener, and effective herbal nanomedicine in the treatment of the recalcitrant bacterial strains and pathogenic fungi like *E. coli* and *Aspergillus niger*.

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