Antimicrobial Activity of Plant Essential oils and Their Emerging Role in Food Sector

[*1]Amita Gaurav Dimri, [1]Dushyant Singh, [1]Rudrangshu Chatterjee
[2]Abhishek Chauhan and [1]M.L Aggarwal
[1]Shriram Institute for Industrial Research, 19, University Road, Delhi-110007, India
[2]Amity Institute of Environmental Toxicology, Safety and Management, Amity University, Sector-125, Noida, India
*Email: pantamita@rediffmail.com

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Abstract-Food preservatives are used to increase the shelf life of food and to maintain the quality for longer time. Natural methods of preservation usually aim to exclude air, moisture, and microorganisms, or to provide environments in which organisms that might cause spoilage cannot survive[25]. In the last scenario, no herbal Ayurvedic preservative had been considered with respect to the use of chemical preservative. Increasing demands for natural and preservative free compounds promoted an idea of the replacement of synthetic preservatives with essential oils having antimicrobial properties.

Essential oils from medicinal plants are potential source of novel antimicrobial compounds especially against food spoilage pathogens. The aim of this project was to compare the antimicrobial activity of essential oils collected from Lemongrass (*Cymbopoga ncitratus)*, Clove *(Syzygiumaromaticum*) and Tulsi (*Ocimum sp*.) against food spoilage organisms.

In this study the essential oil of Lemongrass, Clove and Tulsi were investigated for its activity against Gram negative bacteria *Escherichia coli*, Gram positive bacteria *Micrococcus luteus, Staphylococcus aureus* and *Bacillus cereus, yeast Candida albicans* and fungus *Aspergillus niger, Chaetomium globosum* and *Penicillium funiculosum* using agar well diffusion method. The antimicrobial activity was evaluated by measuring the zone of inhibition. The oils at 30% concentration completely/partially inhibited the growth of food spoilage pathogens. The strongest inhibition activity was observed in Lemongrass oil against all the test organisms; thus it was found more effective as compared to Clove oil and Tulsi oil.

The successful effectiveness of Lemongrass oil could also play a major role in replacing the chemical preservative.

Keywords: *Ocimum, Teniflorum,* Lemon grass and *Cymbopogon citratus*

Introduction

Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens[1].

The use of natural antibiotics agents are the best alternative to synthetic or chemical antibiotics. It prevents development of antimicrobial resistance in bacteria and fungus and also is devoid of side defects. The medical world is on an immense requirement to discover novel antibiotics due to wide spread emergence of resistance among microbial pathogens against currently available antibiotics. However, traditional plants have been proved to be better sources for novel antimicrobial drugs. Growing demands for more natural and preservative-free cosmetics promoted an idea of the replacement of synthetic preservatives with essential oils (EOs) of antimicrobial properties. The antimicrobial effect of essential oil depends on content, concentration and interactions between the main active compounds. Effective preservatives should be characterized by a

broad spectrum of antimicrobial activity at a minimum concentration. Down the ages, numerous essential oils extracted from plant materials have been used for their aroma, flavor, bactericidal, preservative and medicinal properties[2].

Among the medicinal plants, aromatic herbs are a rich source of biologically active compounds useful both in agriculture and medicine[3,4]. Edible, medicinal and herbal plants and spices such as oregano, rosemary, thyme, sage, basil, turmeric, ginger, garlic, nutmeg, clove, mace, savoury and fennel have been successfully used either alone or in combination with other preservation methods[5].

Ocimum tenuiflorum, also known as *Ocimum sanctum*, Tulsi, or Holy Basil from the family Lamiaceae has been described as the "Queen of plants" and the "mother medicine of nature" due to its perceived medicinal qualities [5]. It has been one of the most valued and holistic herbs used over years in traditional medicine in India and almost every part of the plant has been found to possess therapeutic properties[5].

There are 54 volatile components present in Tulsi leaves. The most abundant of the are monoterpenes and sesquiterpenes; in particular monoterpenes such as camphor, cineole, estragol, and eugenol, followed by sesquiterpenes, such as germacrene, caryophyllene, bisabolene. Traditionally, Tulsi is used in different forms; aqueous extracts from the leaves (fresh or dried as powder) are used in herbal teas or mixed with other herbs or honey to enhance the medicinal value. Traditional uses of Tulsi aqueous extracts include the treatment of different types of poisoning, stomach-ache, common colds, headaches, malaria, inflammation, and heart disease[6]. Oils extracted from the leaves and inflorescence of Tulsi have been claimed to have numerous useful properties, including as expectorants, analgesics, anti-emetics, and antipyretics; stress

Figure-1 Leaf of *Ocimum sanctum* and *Ocimum sanctum oil*

reducers and inflammation relievers; and as anti-asthmatic, hypoglycemic, hepatoprotective,hypotensive, hypolipidemic, and immunomodulatory agents[5]. Antioxidants present in Tulsi are significant in the prevention of human illness as free radical scavengers, complexes of pro-oxidant metals, reducing agents and quencher of singlet oxygen formation. Free radicals possess the ability to reduce the oxidative damage associated with many diseases including neurodegenerative diseases, cancer, cardiovascular diseases, cataract and AIDS[7-9]. Antioxidants like Tulsi through their scavenging power are useful for the management of these diseases.

Clove (*Syzygium aromaticum*) on the other hand, is a plant widely cultivated in Spice Islands, Indonesia, Pemba and Zanzibar, though earlier production of the plant was in China.

Figure-2 Leaf of Cloveand Clove oil

This essential oil comprises in total 23 identified constituents, among them eugenol (76.8%), followed by β-caryophyllene (17.4%), α-humulene (2.1%), and eugenylacetate (1.2%) as the main components. Clove oil has biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties, and is used traditionally as a savouring agent and antimicrobial material in food[10-12]. In addition, clove oil is used as an antiseptic in oral infections[13,14]. This essential oil has been reported to inhibit the growth of moulds, yeasts and bacteria[15]. It was effective against *L. monocytogenes* and *S. Enteritidis* in Tryptone Soya Broth and cheese[16]. The high levels of eugenol contained in clove essential oil are responsible for its strong biological and antimicrobial activities. It is well known that both eugenol and clove essential oil phenolic compounds can denature proteins and react with cell membrane phospholipids changing their permeability and inhibiting a great number of Gram-negative and Gram-positive bacteria as well as different types of yeast[17,18]. It is used in the seasoning of food.

Cymbopogon citrates commonly known as lemongrass is an herb which belongs to the grass family of *Poaceae*. It is utilized for its distinct lemon flavour and citrusy aroma. It is a tall, perennial grass which is native to India and tropical regions of Asia. It is a coarse and tufted plant with linear leaves that grows in thick bunches, emerging from a strong base and standing for about 3 meters in height with a meter-wide stretch.

The genus *Cymbopogon* comprises of 55 species of grasses, two of which are referred to as lemongrass. These are *Cymbopogon citratus,* which is famously preferred for culinary use and *Cymbopogon flexuosus,* which is used in the manufacturing of fragrances because of its extended shelf life, owing to the low amount of myrcene in that variety.

Lemongrass is widely used as an essential ingredient in Asian cuisines because of its sharp lemon flavour. Lemongrass oil used as a pesticide and preservative, is put on the ancient palm-leaf manuscripts found in India as a preservative.

One of the main constituents of the many different species of lemongrass (genus *Cymbopogon*)is citral (3,7-dimethyl-2,6-octadien-1-al). Lemongrass oil has been found to contain up to 75-85% citral. Lemongrass also contains z-citral, borneol, estragole, methyleugenol, geranyl acetate (3,7-dimethyl-2,6-octadiene-1-ol acetate), geraniol (some species higher in this compound than citral), beta-myrcene (MYR, 7-methyl-3-methylene-1,6 octadiene), limonene, piperitone, citronellal, carene-2, alpha-terpineole, pinene, farnesol, proximadiol, and (+)-cymbodiacetal. The volatile oil from the roots contains 56.67% longifolene-(V4) and 20.03% selina-6-en-4-ol.

Figore -3 Leaf of lemongrass and lemongrass oil

In particular, a study of *Cymbopogon martinii* isolated fatty acids, common sterols, and 16-hydroxypentacos-14(z)-enoic acid*Cymbopogon citratus* has been cultivated over many years for medicinal purposes in different countries throughout the world. Lemongrass is an aromatic storehouse of essential nutrients providing an array of health benefits. It is a source of essential vitamins such as vitamin A, vitamin B1 (thiamine), vitamin,B2 (riboflavin), vitamin B3(niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), folate, and vitamin C. It also provides essential minerals such as potassium, calcium, magnesium, phosphorous, manganese, copper, zinc and iron, which are required for the healthy functioning of the human body. It offers no harmful cholesterol or fats. The use of lemongrass was found in folk remedy for coughs, consumption, elephantiasis, malaria, ophthalmia, pneumonia and vascular disorders. Researchers have found that lemongrass holds antidepressant, antioxidant, antiseptic, astringent, bactericidal, fungicidal, nervine and sedative properties[19]. It can be used in cleaning wounds and treatment of skin diseases such as ringworm. It can also be used in food poisoning, staphylococcal infections, and other common infections.

The oil has been found to possess bactericidal and anti-fungal properties, which is comparable to penicillin in its effectiveness[20]. As a bactericidal agent, the lemongrass oil was found to be effective against many bacterial species including *Acinetobacter baumanii*, *Aeromonas veronii*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella enterica*, *Serratia marcesens*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Corynebacterium equii*, *Staphylococcus aureus* and so on[21-24].

Antimicrobial activity of the *Cymbopogon citratus* (lemongrass) essential oil against food-borne pathogens was determined to investigate its potential for reducing microbial population of food products. Previous reports suggest that lemongrass essential oil is a safe natural flavour complex, preservative, and food spoilage inhibitor capable of reducing the risk of diseases associated with contaminated products.

The aim of our study was to gain the attention on usage of essential oils as antimicrobial agents and we use lemongrass oil against a wide spectrum of food spoilage pathogenic two strain of Gram negative bacteria namely; *Escherichia coli,* and three strain of Gram positive bacteria namely; *Micrococcus luteus, Bacillus cereus, Staphylococcus aureus, and against some fungus namely Candida albicans, Aspergillus niger Penicillium funiculosum and Chaetomium globosum.*

Material and Methods

Lemongrass oil, Clove oil and *Ocimum* oil was investigated for activity against various selected microorganisms. The material and methods needed for this study are listed below in accordance to their source of availability and grades.

Media and Chemicals

- Nutrient Agar(NA)
- Mueller Hinton Agar (MHA)
- Chloramphenicol Yeast Glucose Agar (CYGA)
- Antibiotic Assay Medium 11
- Antibiotic Assay Medium 19
- Sodium Chloride
- Tween-80

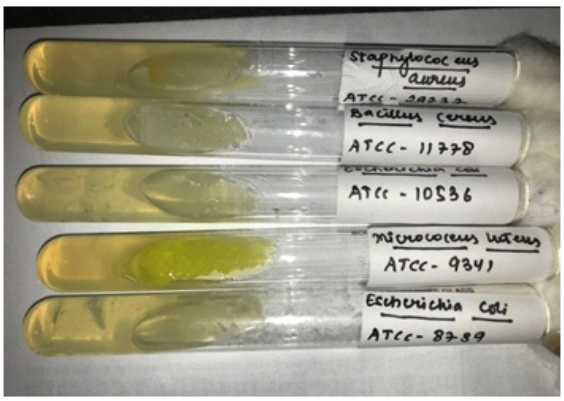
Procurement of lemongrass oil

The essential oil of lemongrass was procured from SIGMA LRICH, India.

Fungal and Bacterial strains

The test organisms used in this study was taken from Department of Microbiology of Shriram Institute for Industrial Research New Delhi as a standard strain and the following details are given below.

Bacterial strain	ATCC No.
Micrococcus luteus	9341
Escherichia coli	8739
Escherichia coli	10536
Staphylococcus aureus	29737
Bacillus cereus	11778



Fungal strain	ATCC No.
Aspergillus niger	16404
Candida albicans	3471
Chaetomium globosum	6205
Penicillium funiculosum	11797

Figure- 4 Bacterial cultures used in study

Figure - 5 Fungal cultures used in study

Propagation and maintenance of test organisms

The bacterial test organisms were streaked on the Nutrient Agar slants and were incubated overnight at 37˚C and fungus test organisms were streaked on the Chloramphenicol Yeast Glucose Agar slants and were incubated for 5 days at 22˚C.

Preparation of concentrations of oil

The 30% concentrations (v/v) ofoil were prepared aseptically in sterile tween-80.

Antimicrobial activity

The testing of the bacterial and fungal cultures for the inhibitory effect of essential oil was done at 30% concentrationby using agar well diffusion method.

Agar Well Diffusion Assay (Zone of Inhibition Evaluation): Antibiotic susceptibility and resistance were evaluated by agar well diffusion assay. 0.5 McFarland density of bacterial and fungal culture was adjusted using normal saline (0.85% NaCl) using densitometer to get bacterial and fungal population of 1.0 x 10^8 cfu/ml. 100µl of each

of the adjusted cultures were mixed into separate 100 ml of sterile, molten, cool MHA (Meuller-Hinton agar), mixed well and poured into sterile Petri plates. These were allowed to solidify and then individual plates were marked for each individual isolates. Each plate was punched to make wells of 6 mm diameter with the help of sterile cork borer at different sites of the plates. 100 µl of respective essential oil were pipette out into the well in assay plates. Bacterial plates were incubated overnight at 37°C and fungal plates were incubated for 5 days at 22°C. Following incubation, petri-plates were observed for the inhibition zones, diameters of which were measured by using Vernier Callipers.

Results and Discussion

Lemongrass oil at concentration of 30% was found the most effective essential oil against all the selected microorganisms as compared to Clove oil and Ocimum oil. All the selected microorganisms showed difference in their sensitivity against three different essential oils. The strongest inhibition activity was observed in Lemongrass essential oil against *Bacillus cereus* and *Candida albicans*. Results of antimicrobial activity of essential oils using agar well diffusion method revealed that Lemongrass is much effective and broad spectrum of antimicrobial activities as compared to Clove oil and Tulsi oil against food spoilage organisms.

Our observation revealed that only one strain of bacteria was resistant against Lemongrass while remaining all microorganisms were sensitive.

Table -1 Antimicrobial activity of lemongrass oil against various selected microorganisms-

S. No.	Name of microorganisms	ATCC No.	Zone of inhibition (30%)		Average
1.	*Escherichia coli*	8739	15.80 13.48	12.80 13.45	13.88
2.	*Escherichia coli*	10536	NZO	NZO	0.0
3.	*Micrococcus luteus*	9341	18.51 21.98	21.09 20.30	20.47
4.	*Bacillus cereus*	11778	20.21 18.99	18.16 20.29	19.41
5.	*Staphylococcus aureus*	29737	14.08 15.90	27.03 21.22	19.56
6.	*Aspergillus niger*	16404	50.08 51.06	50.36 49.08	50.15
7.	*Candida albicans*	3471	35.63 35.38	39.63 38.08	37.17
8.	*Chaetomium globosum*	6205	36.21 35.27	33.84 33.65	34.73
9.	*Penicillium funiculosum*	11797	15.47 13.89	16.32 14.98	15.17

*NZO No zone observed

*Zone of inhibition in mm. Diameter including well diameter of 6.0mm.

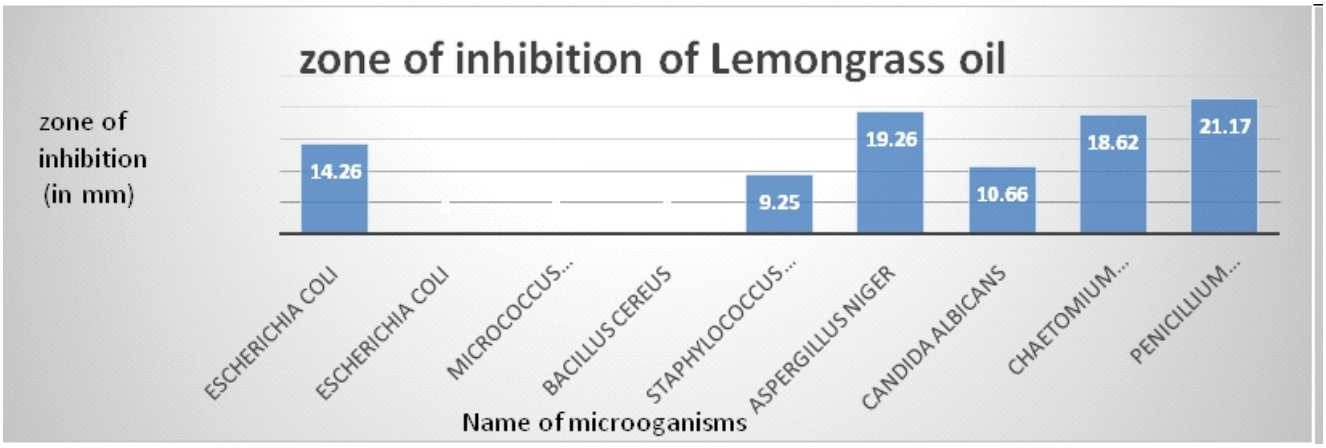


Figure -6 Zone of Inhibition of Lemon Grass Oil

Table-2 Antimicrobial activity of clove oil against various selected microorganisms-

S. No.	Name of Microorganisms	ATCC No.	Zone of inhibition (30%)		Average
1.	*Escherichia coli*	8739	11.31 10.37	11.00 11.58	11.07
2.	*Escherichia coli*	10536	11.24 11.00	13.65 15.31	12.8
3.	*Micrococcus luteus*	9341	17.75 19.19	9.87 11.40	14.55
4.	*Bacillus cereus*	11778	15.07 14.23	13.89 12.23	13.86
5.	*Staphylococc us aureus*	29737	9.27 8.82	10.95 10.04	9.77
6.	*Aspergillus niger*	16404	34.43 43.23	46.32 42.25	41.56
7.	*Candida albicans*	3471	17.79 19.92	16.90 17.93	18.16
8.	*Chaetomium globosum*	6205	40.28 40.56	37.01 40.30	39.53
9.	*Penicillium funiculosum*	11797	35.18 37.50	45.59 43.10	40.34

*NZO No zone observed
*Zone of inhibition in mm. Diameter including well diameter of 6.0mm.

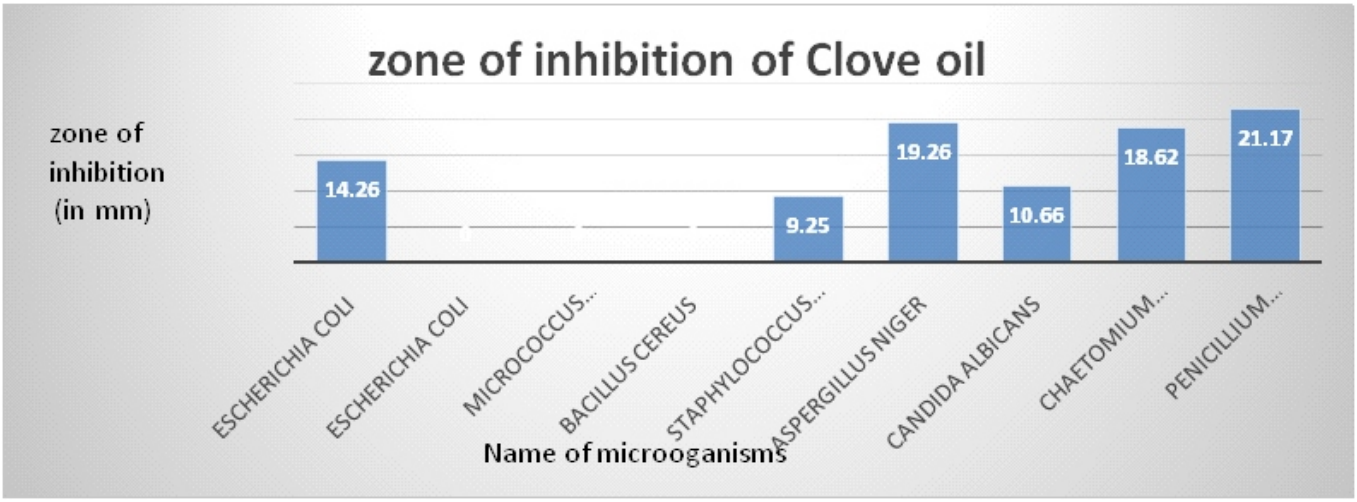


Figure-7 Zone of inhibition of Clove Oil

Table-3 Antimicrobial activity of *Ocimum* oil against various selected microorganisms-

S.No.	Name of microorganisms	ATCC No.	Zone of inhibition (30%)		Average
1.	*Escherichia coli*	8739	15.38 13.95	13.42 14.29	14.26
2.	*Escherichia coli*	10536	NZO	NZO	0.0
3.	*Micrococcus luteus*	9341	NZO	NZO	0.0
4.	*Bacillus cereus*	11778	NZO	NZO	0.0
5.	*Staphylococc us aureus*	29737	10.12 9.24	9.12 8.50	9.25
6.	*Aspergillus niger*	16404	19.79 19.54	20.98 16.76	19.26
7.	*Candida albicans*	3471	9.85 10.44	11.47 10.86	10.66
8.	*Chaetomium globosum*	6205	18.59 18.27	19.13 18.47	18.62
9.	*Penicillium funiculosum*	11797	20.84 19.41	21.84 22.58	21.17

*NZO No zone observed
*Zone of inhibition in mm. Diameter including well diameter of 6.0mm.

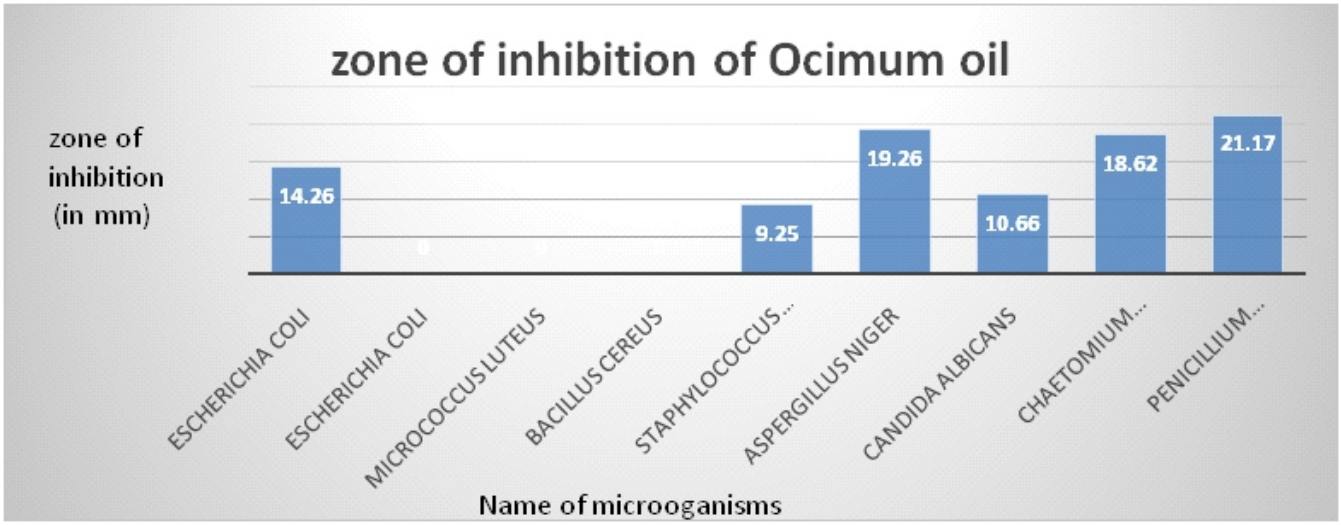


Figure-8 Zone of inhibition of Ocimum o

Conclusion

The major concern is extensive uses of chemical food preservatives. The present scenario shows overuse of chemical preservatives in food products, this may not only lose the natural property of food but also effect the consumer's health.

Increasing demands for natural and preservative free compounds promoted an idea of the replacement of synthetic preservatives with essential oils of antimicrobial properties

The comparative effects of essential oils on the various test organisms are demonstrable indications of the oil as an antimicrobial agent. Thus a study had been carried out to show that an herbal product Lemongrass oil is much potential against food spoilage organisms as compared to Clove and Tulsi oil.

The results conclude that Lemongrass Oil, Clove Oil and Tulsi Oil possesses inhibitory activity in the series Lemongrass Oil > Clove Oil >Tulsi

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