Research Article

Extraction of Active Components from Crude Ocimum Sanctum and Maintenance of C. Elegans

Amrita Mishra*, Dr. Rakhi Agarwal[1]

1.Associate Professor, IFS, GFSU Worked at laboratory of analytical and molecular toxicology Institute of forensic science gujarat forensic sciences university, Near DFS Head Quarter, Sector 9, Gandhinagar, Gujarat, India.

***Corresponding Author: Amrita Mishra**, M.Sc. Forensic Science, Specialization in Forensic Chemistry Toxicology.

Received Date: February 5, 2021

Publication Date: March 01, 2021

Abstract

Ayurveda is the traditional Indian system of medicine that is meant for curing diseases and also in preventing the occurrence of diseases. It focuses on healthy lifestyle practices and regular consumption of herbs especially mediational plants for curing various diseases. In the traditional system of medicine, different parts of the Tulsi plant (Ocimum sanctum Linn.) possess various therapeutic effects. Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituents present in O. sanctum L. is largely responsible for the therapeutic potentials.

Caenorhabditis elegans is a free-living, transparent nematode, about 1mm in length, that lives in the temperate soil environment. This has been widely used as a model organism for various studies. The effect of crude extract of Ocimum sanctum on C. elegans has been studied here. Firstly, it includes extraction process from Tulsi leaves, then the maintenance of C. elegans which involves Preparation of NGM agar plates, M9 buffer solution, MEM for E. coli; seeding and Chunking of plates; age synchronization.

The study is basically on the effect of crude extract of Ocimum on C. elegans. It shows the antioxidant capacity, resistance against thermal stress, protective and life span extending properties of the medicinal plant (Tulsi) in C. elegans.

Introduction

Ayurveda

The word "Ayurveda" comes from the Sanskrit language which means "life-knowledge". Ayurveda is a system of medicine that has been used for several millennia. Its therapies are based on complex herbal compounds, minerals, and metallic substances. While Ayurveda is considered a complementary and alternative form of medication in the modern world, it is the only medicine and therapeutic therapy in many rural villages.

Ayurveda names three elemental and constitutional substances or "energies" called *doshas* which are *Vata, pitta,* and *Kapha*, which loosely translates to air, fire, and water, respectively. *Doshas* and its balance in the human body is the emphasis on maintenance of health and treatment of disease in the Ayurvedic system of medicine. Medicinal plants that have been focused on Ayurveda include – Neem **(***Azadirachta indica***)**, Guggul **(***Commiphora weight***)**, Tulsi **(***Ocimum uniform***)**, Amla- Indian gooseberry **(***Emblica Officinalis***)**, Turmeric/ Haldi **(***Curcuma longa***)**. **(**Rupani & Chavez, 2018**)**

Figure 1: Ayurvedic medication – Herbal extracts and products

The ethno pharmacological relevance of Ayurveda entails a scientific tradition of harmonious living and its origin is contained in Rigveda and Atharvaveda. Ayurveda is a traditional healthcare system of Indian medicine since time immemorial. Several Ayurvedic medicines have been exploiting for the management and treatment of various diseases in human beings. Hence, Ayurveda has now become a modern practice and can be referred to as "*Tradition to Trend*". The potential of Ayurvedic medicine needs to be explored and validation approaches for better therapeutic leads. **(**Mukherjee et al., 2017**)**

Ayurveda uses the natural elements or components to eliminate the root cause of a disease by restoring balance and also creates a healthy lifestyle to prevent the reoccurrence of imbalance. Herbal medicines have existed worldwide with a long record history and were used in ancient Chinese, Greek, Egyptian, and Indian medicine for various therapeutic purposes. World Health Organization estimated that 80% of the world's inhabitants still rely mainly on traditional medicines for their healthcare.

The Indian subcontinent is well-known for its major biodiversity with about 45,000 plant species. About 15,000 medicinal plants have been recorded in India, out of which the communities use 7,000 to 7,500 plants for therapeutic purposes against various diseases. In Ayurveda, single or multiple herbs **(**polyherbal**)** are used for treatment purposes. The Ayurvedic literature "Sarangdhar Samhita" highlighted the concept of polyherbal to achieve greater therapeutic efficiency.

A combination of certain plant extracts may improve the therapeutic efficiency. The active phytochemical constituents of individual plants are sometimes insufficient to achieve the desired therapeutic effects, Hence, when multiple herbs are combined in a particular ratio, it may result in a better therapeutic effect and reduce the toxicity. This concept is known as polyherbal. **(**Parasuraman et al., 2014**)**

In this particular thesis, we are going to study one Ayurvedic herb i.e. Tulsi **(***Ocimum sanctum***)**, and its effects on the living organism by using Caenorhabditis elegans as a model organism. C. elegans shows certain behavioral changes when exposed to an environment containing either a toxic substance or a medicinal substance. Hence, it is one of the best-preferred model organisms for such kind of studies that involves any toxin or a medicinal compound. Also, as it is microscopic, it is quite easy to grow and use for experimental purposes without violating ethics.

Tulsi (ocimum sanctum/ocimum tenuiflorum)

[queen of herbs]

Scientific classification

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Asterids

Order: Lamiales

Family: Lamiaceae

Genus: Ocimum

Species: tenuiflorum/sanctum

Figure 2: *Ocimum sanctum* – Tulsi

Ocimum sanctum, commonly known as Holy basil or Tulsi is a perennial plant belonging to the family Lamiaceae. It is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics.

Tulsi is cultivated for religious and traditional medicinal purposes and its essential oil. It is widely used as an herbal tea and is commonly used in Ayurveda medicine. It is also used by devotes **(**Hindu**)** for worship purposes.

Morphology

Tulsi is an erect, multibranched subshrub, 30-60 cm **(**12-24 inch**)** tall. Leaves are simple, opposite green or purple colored that are strongly scented and hairy stems. Leaves have petiole and are ovate, up to 5cm long. Flowers are purplish.

Ocimum Sanctum Genome

The draft assembly of Krishna Tulsi genome is about 386 Mb [1 Mb = 1,000,000 bp]. The plastid **(**Chloroplast**)** genome is about 1,42,524 base pairs. **(**Rastogi et al., 2015**).**

The pathway leading to the production of medicinally significant phytochemicals in O. sanctum is similar to that of *Arabidopsis thaliana* and certain other plants. Expression levels of anthocyanin biosynthesis-related genes in leaf samples of Krishna Tulsi were observed to be relatively high, explaining the purple coloration of the leaves. **(**Upadhyay et al., 2015**)**

Significance in Hinduism

Tulsi, the 'Queen of herbs', the legendary 'Incomparable one' of India, is one of the holiest and most cherished of the many healing and health giving herbs of the orient. The sacred basil is renowned for its religious and spiritual sanctity and has an important role in the traditional Ayurvedic and Unani system of holistic health and herbal medicine of the east.
Within Ayurveda, Tulsi is known as:

- The Incomparable one
- Mother Medicine of Nature
- The Queen of Herbs

It is also referred to as *the Elixir of life* for both its medicinal and spiritual properties. The emerging science on Tulsi, which reinforces ancient Ayurvedic wisdom, suggests that Tulsi is a tonic for the body, mind, and spirit that offers solutions to many modern-day health problems.

Divine Tulsi

In Hinduism, Tulsi is worshiped as a goddess and every part of this plant is considered sacred. Even the surrounding soil, which has been found to harbor beneficial endophytic fungi, is considered as an aspect of the divine. Hindu households are considered incomplete without a Tulsi plant, which is typically ornated in an earthen pot situated in a courtyard where it serves both practical and ceremonial purposes.

Figure 3: Indian women offering water to Tulsi Plant at courtyard

Types of Tulsi

(Jurges et al., 2018**)**

3 main types of Tulsi plant are:

1. Rama Tulsi
2. Krishna Tulsi
3. Vana Tulsi

Rama Tulsi

Rama Tulsi is also called Sri Tulsi. It is the most dominant form of all having green colored leaves with light purple flowers. It is used mostly for religious purposes and is usually termed as Ocimum tenuiflorum. It has a clove-like scent, due to the chemical Eugenol.

Krishna Tulsi

The purple-leafed Krishna Tulsi, named after it resembles the color of the Hindu deity Krishna, is preferred for medical use and also assigned as O. uniform L. It has a clove-like aroma and peppery flavor. Krishna Tulsi is the right medicinal Tulsi as it has more potency. It has also been used as a traditional contraceptive.

Vana Tulsi

Vana Tulsi, meaning 'forest' Tulsi may indicate that it is often found in the wild. The wild leaves of it are bright or light green. They grow in the wild and are indigenous to many areas of Asia and northeast Africa.

Figure 4: Three main types of Tulsi plant

Apart from there three main types, there is a fourth type of Tulsi known as Kapoor Tulsi. However, over 60 different species are belonging to the genus Ocimum tenuiflorum distributed all over the world.

Varieties of Tulsi mostly found in the Indian Subcontinent are Amrita Tulsi **(** Amrita meaning immortality, it is called so because it is very difficult to kill this plant as it can grow in toughest and variety environment**)**, sweet Basil, Lime Basil, Greek Basil, Thai Basil, Cinnamon Basil or Mexican Basil, Lettuce leaf Basil, Christmas Basil, Napoletano Basil, Summerlong Basil, Genovese Basil, Ararat Basil, etc.

Chemical composition of ocimum sanctum:

(Verma, 2016**)(**Cohen, 2014**)**

Phytochemical constituents of Tulsi includes:

-Eugenol

-Oleanolic Acid

-Ursolic Acid

-Rosmarinic Acid

-Carvacrol

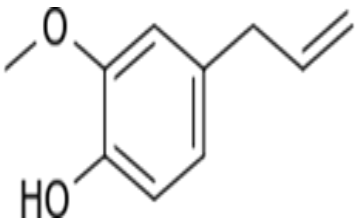
-Linalool

-β- Caryophyllene

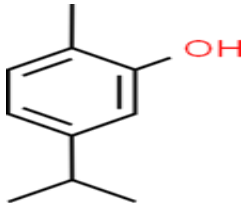
Essential oil consists mostly:

- Eugenol **(**~70%**)**
- β-Caryophyllene **(**~11.0%**)**
- Germacrene **(**~2%**)**

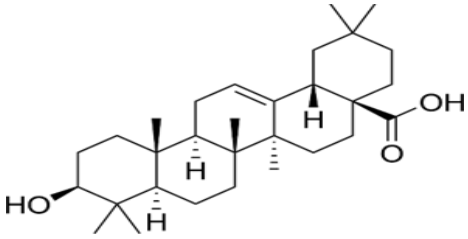
With the balance being made up of various trace compounds, mostly terpenes.



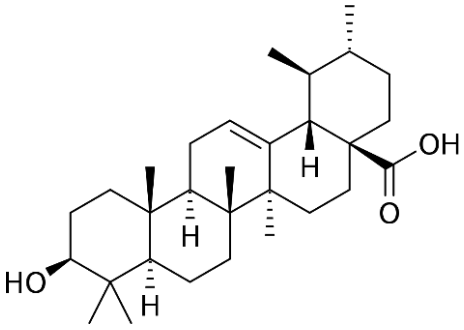
Eugenol



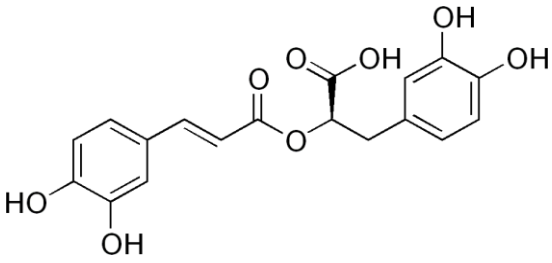
Carvacrol



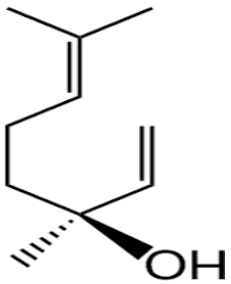
Oleanolic Acid



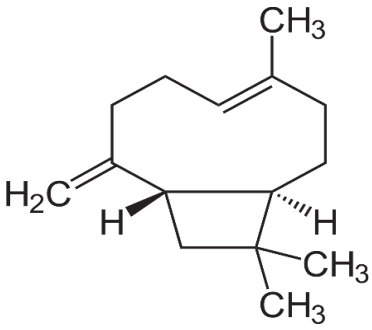
Ursolic Acid



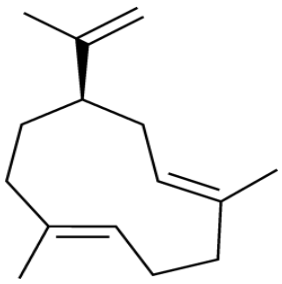
Rosmarinic Acid



Linalool



β – Caryophyllene



Germacrene

Figure 5: Structures of Phytochemical constituents of Tulsi

Caenorhabditis Elegans: A Model Organism

Scientific Classification

Kingdom: Animalia

Phylum: Nematoda

Class: Chromadorea

Order: Rhabditida

Family: Rhabditida

Genus: Caenorhabditis

Species: Elegans

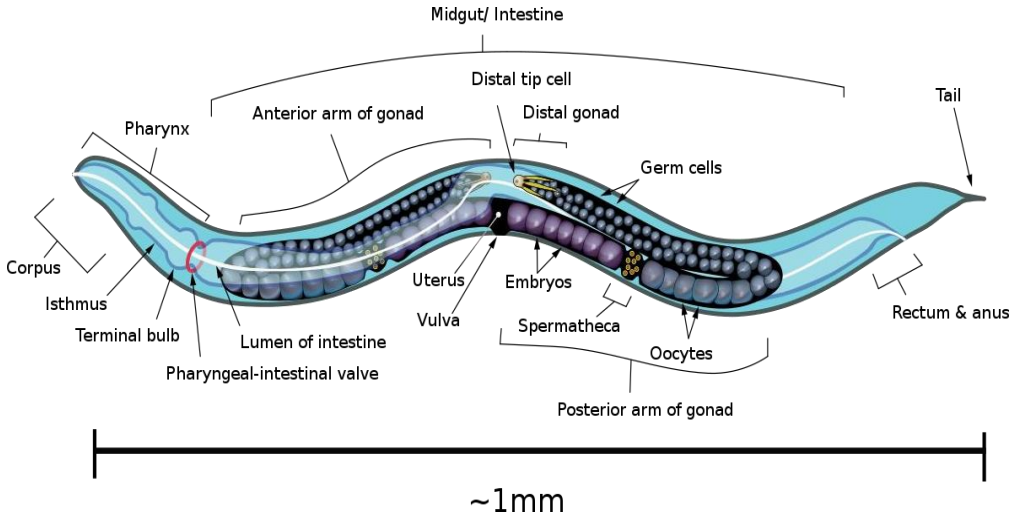


Figure 6: Adult Caenorhabditis elegans containing eggs

Caenorhabditis elegans, commonly known as roundworm, is a small, free-living, transparent nematode, about 1 mm in length, that lives in temperate soil environments. The name is a blend of the Greek word casino, meaning recent; habits, meaning rod-like and Latin elegans, meaning elegant.

C. elegans normally lives in soil and eats bacteria such as E. coli. Worms contain rudimentary feeding, neural, and reproductive systems. Caenorhabditis elegans can be grown easily in laboratories and can be frozen for long-term storage.

C. elegans contains 302 neurons and harbors approximately 20,470 protein-coding genes. It was the first multicellular eukaryote to have its complete genome sequenced.

C. elegans is an unsegmented pseudocoelomate and lacks respiratory or circulatory systems. Most of them are hermaphrodites and a few are males. Males have specialized tails for a mating that include spicules.

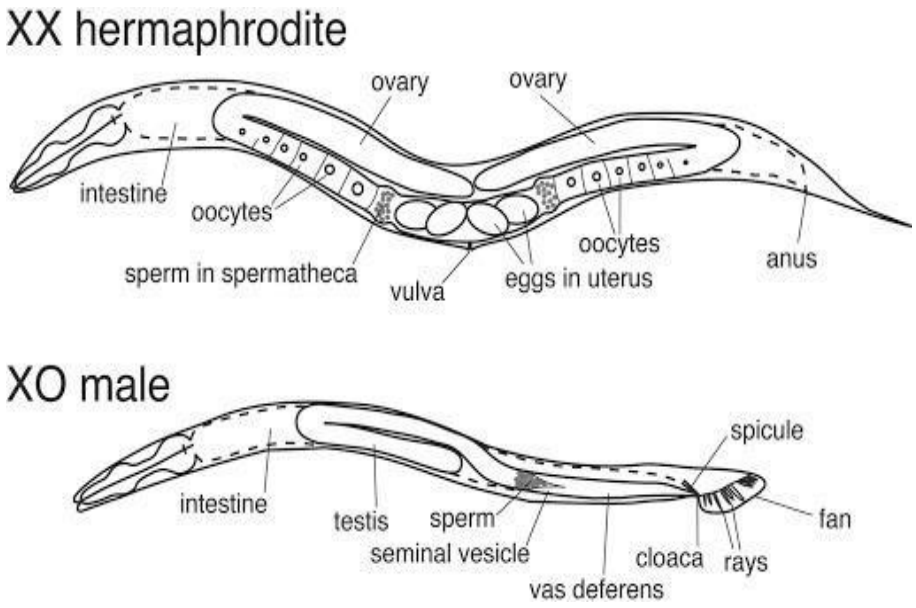


Figure 7: Hermaphrodite and male C. elegance

C. elegans has become established as a standard model organism for a great variety of genetic investigations, and has been useful for studying developmental biology, cell biology and neurobiology. In 1963, Sydney Brenner proposed research into the molecular and developmental biology of C. elegans, which have been extensively used as a model organism. It was the first multicellular organism to have its whole genome sequenced, and as of 2018, is the only organism to have its connectome **(**neuronal "wiring diagram"**)** completed.

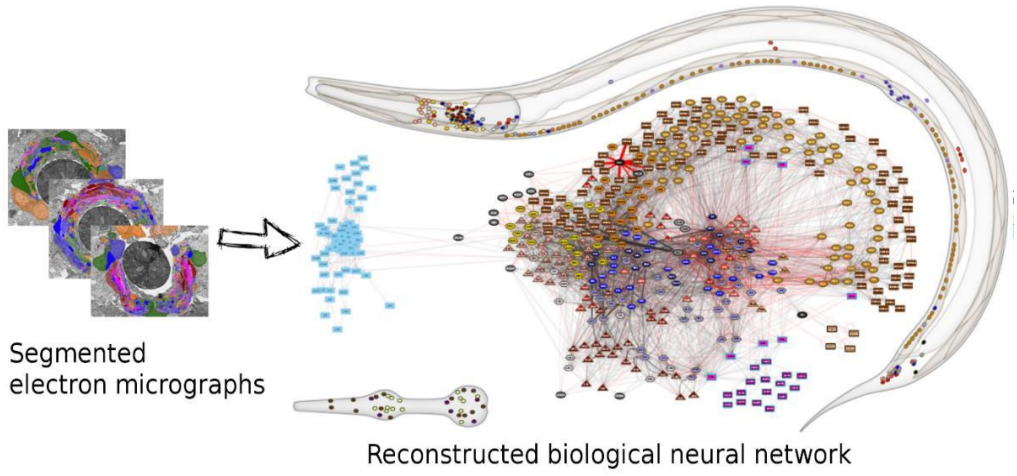


Figure 8: Neuronal wiring diagram- Connectome

Elegans connectomes are an information processing network that receives input from about 90 sensory neurons, process that information through a highly recurrent network of about 80 interneurons, and it produces a coordinated output from almost 120 motor neurons that control the nematode's muscles.

Life cycle of C. Elegans

(Organism, 2014**)**

Similar to other nematodes, the life cycle of C. elegans is comprised of:

- The embryonic stage
- Four larval stages **(**L1, L2, L3, L4**)**, and
- Adulthood

The end of each larval stage is marked with a molt where a new, stage-specific cuticle is synthesized and the old one is shed. While larval shift occurs, the cuticular lining of the pharynx breaks down into the intestine at the posterior and through the mouth at the anterior. The larva further pushes against the old cuticle and makes a hole at the head region through which it emerges. The larva then starts to feed immediately. In this way, the next larval stage is achieved.

Out of all four larval stages, L3 is the most preferred stage for the behavioral study of the organism when exposed to certain substances.

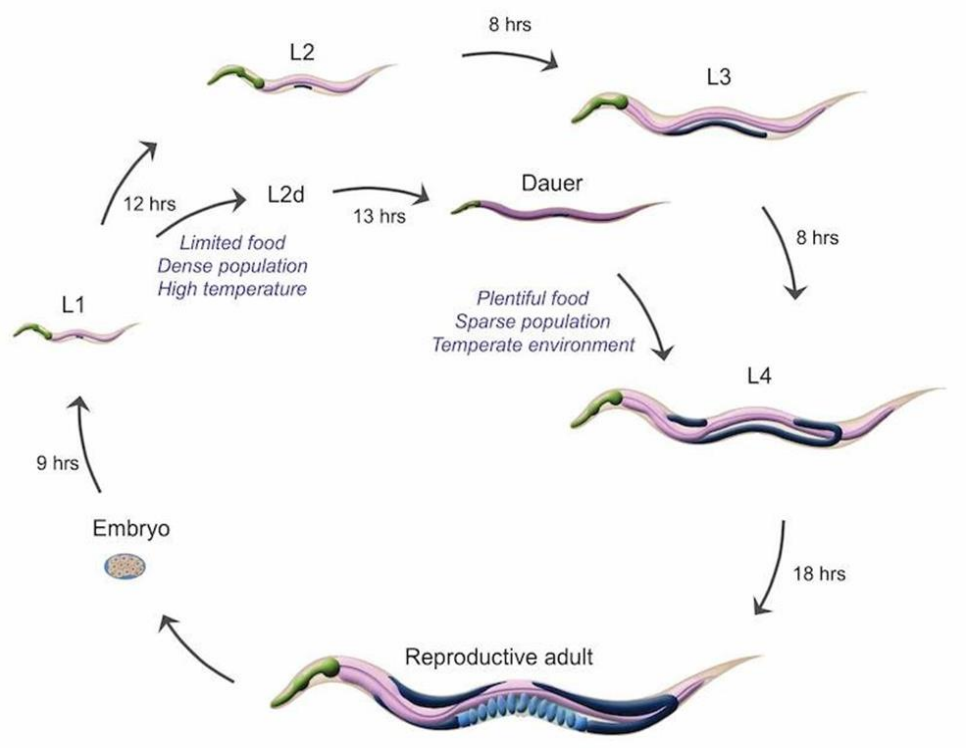


Figure 9: Life cycle of Caenorhabditis elegance

C. Elegans as a Model Organism

In 1965, Sydney Brenner settled on Caenorhabditis elegans as a model organism to study animal development and behavior for reasons that are now well known **(**Brenner,1973, 1988**)**.

This soil nematode has a great potential for genetic analysis, partly because of its rapid **(**3- day**)** life cycle, small size **(**1.5-mm-long adult**)**, and ease of laboratory cultivation. Thousands of animals can be grown on a single petri dish seeded with a lawn of Escherichia coli as the food source. Their mode of inbreeding is by the self-fertilizing hermaphrodite, also combined with the ability to cross hermaphrodites with males.

Other key features include nematode's small genome **(**only 20 times that of E. coli**)** and anatomical simplicity **(**>1000 cells**)**, including the 302-cell hermaphrodite nervous system. With a nervous system that small, Brenner proposed that its complete circuitry could be determined by serial-section electron microscopy, a vision realized 20 years later. The ultimate goal was to determine the role of each gene involved in neural development and function.

An important reason C. elegans was chosen for the study was that high-quality electron micrographs had been obtained from specimens of this species by Nichol Thomson, who was hired by Brenner in October 1964.

Other investigators characterized responses to chemoattractant and the sensory ultra-structure of the wild type and mutants defective in chemotaxis. Ultimately, the wild-type reconstructions showed all the connections of all the neurons in the hermaphrodite nervous system. Analysis of the wild-type circuitry allowed detailed models of how neurons function together to generate behavior. In recent years, the wild-type circuit diagram has provided the foundation for interpreting the phenotypes of behavioral and locomotory mutants and the spatial deployment and function of neurotransmitters.

Review of Literature

Ayurveda focuses on healthy lifestyle practices and regular consumption of adaptogenic herbs. The practice includes consumption of fresh, minimally processed foods, the use of Rasayanas **(**formulas**)** that work against aging and diseases, including detoxification.

Regular consumption of adaptogenic herbs enhances the body's capacity to maintain balance in the presence of stressors.

Out of all herbs used in Ayurveda, Tulsi **(**Ocimum sanctum Linn**)** is preeminent and has several beneficial effects. The cultivation of Tulsi plants has both spiritual and practical significance. There are pieces of evidence that Tulsi can address physical, chemical, metabolic, and psychological stress through a unique combination of pharmacological actions.

Tulsi has been found to protect organs and tissues against heavy metals, chemical stress from industrial pollutants, physical stress from prolonged physical exertion, ischemia, physical restraint, and exposure to cold and excessive noise. Tulsi also has the property to counter metabolic stress by normalizing the blood glucose level, blood pressure, and lipid levels. It can counter psychological stress through positive effects on memory and cognitive function and it's an anxiolytic and anti-depressant properties. It is clinically efficient and safe for human consumption. **(**Jamshidi & Cohen, 2017**)**

Tulsi's antimicrobial activity which includes activities against a range of human and animal pathogens, suggests that it can be used as a hand- sanitizer, mouth wash, and water purifier as well as in animal rearing, wound healing **(**Shetty et al., 2008**)**, preservation of foodstuffs and herbal raw materials,

etc.(Yamani et al., 2016)

Toxicological study of o. sanctum leaf extract reveals that it is safe for human consumption and use. (Gautam & Goel, 2014)

The use of Tulsi in daily rituals is a testament to Ayurvedic wisdom and provides an e.g. of ancient knowledge offering solutions to modern problems.

Tulsi: A Potent Adaptogen

(Lahon & Das, 2011)

In Ayurveda, Tulsi is known as 'The Incomparable one', 'Mother Medicine of Nature', 'The Queen of Herbs', and 'Elixir of life' for both its medicinal and spiritual properties. Daily consumption of Tulsi is said to prevent disease, promote general health, wellbeing, and longevity and assist in dealing with the stress of daily life. Tulsi is also found to be credited for giving luster to the complexion, sweetness to the voice, and fostering beauty, intelligence, stamina, and a calm emotional disposition.

In addition to health-promoting properties, Tulsi is recommended as a treatment for a range of conditions including anxiety, cough, asthma, diarrhea, fever, dysentery, arthritis, eye diseases, otalgia, vomiting, hiccups, indigestion, gastric, cardiac, and genitourinary disorders, skin diseases, ringworm, back pain, insect bite, snake and scorpion bites, and malaria.

As a potent adaptogen, Tulsi has a unique combination of pharmacological actions that promote wellbeing. The medicinal properties of Tulsi have been studied in hundreds of scientific studies including in vitro, animal and human experiments which reveal that Tulsi has a unique combination of actions that include – Antimicrobial (including antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, anthelmintic), Anti-diarrheal, Anti-oxidant and anti-diabetic (Ononamadu et al., 2019) , Anti-asthmatic, Anti-cataract, Anti-inflammatory, Anti-carcinogenic (Bhat et al., 2015), Anti-pyretic, Anti-allergic, Anti-tussive, Chemopreventive, Radioprotective, Hepato-protective(Guest & Grant, 2016).

Neuroprotective (Bhattacharyya & Bishayee, 2013), (Hening et al., 2018) Mosquito repellent, Cardio-protective (Fathiazad et al., 2012) , Analgesic, Immunomodulator, Central Nervous System(CNS) depressant, Memory enhancement, Diaphoretic, Anti-arthritic, Anti-spasmodic, Anti-emetic, Anti-ulcer, Anti-fertility,Male contraceptive(Sethi et al., 2010) , Anti-thyroid, Anti-stress, Adaptogenic, Anti-leukoderma, Anti-coagulant activities.O.

Sanctum stimulates the expression of choline acetyltransferase on cerebral microvascular endothelial cells in humans. **(**Kusindarta et al., 2016**)** These pharmacological actions help the body and mind cope with a wide range of chemical, physical, infections, and emotional stresses and restore physiological and psychological function.

Ocimium Sanctum against Mercury

(Bhattacharyya & Bishayee, 2013**)**

Mercury is used in many places such as industry, agriculture, medicine, and other fields. On exposure, it affects the nervous, cardiovascular, pulmonary, gastrointestinal, and renal systems, also the embryo. Inorganic mercury is mostly deposited in the kidney, which is the target organ, and exerts its toxicity there. There are various investigations reporting protection against mercury-induced toxicity by Ocimum sanctum taking Swiss albino mice as a model organism.

Various combination of mercury as mercuric chloride and Ocimum sanctum was administered to the mice at different time. And the activity of alkaline phosphatase **(**ALP**)**, acid phosphatase **(**ACP**)**, lactate dehydrogenase **(**LDH**)**, and lipid peroxidation **(**LPO**)** was measured in the kidney homogenates. A result from the study suggested that pre and post treatment of Ocimum sanctum leaves extract can significantly protect the renal damage against mercuric chloride- induced toxicity. Ocimum sanctum is also effective in reducing the pathological alteration in the kidney.

Several types of research show that various species of plant kingdom have the potential for clinical management of mercury toxicity in humans. **(**Bhattacharya, 2018**)**.

Tulsi- A Medicinal Plant for Prevention and Treatment of Cancer

(Ramesh & Satakopan, 2010**)**

Ocimum sanctum, commonly known as Tulsi or Holi Basil has several therapeutic properties due to its number of phytoconstituents which have exceptionally beneficial effects. There is also evidences that this plant has preventive or therapeutic properties for oncologic disease conditions.

Banerjee et al. suggested that Ocimum sanctum leaf extract or its bioactive component has chemopreventive potential against chemical carcinogenesis. Oral administration of alcoholic extract of leaves of Ocimum sanctum significantly elevates the activity of hepatic Cytochrome P-450, Cytochrome b5, aryl hydrocarbon hydroxylase **(**AHH**)**, and GST and increased the GSH content in the liver of the mice.

Antitumor effects of a botanical extract of Ocimum sanctum have been examined by several investigators using various established tumor models **(**Xenograft cancer models**)**. Oral administration of aqueous and ethanolic leaf extract to mice bearing sarcoma-180 solid tumors resulted in a significant reduction in tumor volume and an increase in the survival of tumor-bearing animals.
Ocimum species have been found to possess the potential for inhibiting breast tumor growth and angiogenesis. **(**Nangia-Makker et al., 2007**)**

Holy Basil leaf extract has a potential role in therapy by decreasing tumorigenicity and metastasis of aggressive pancreatic and cancer cells in humans. **(**Shimizu et al., 2013**)**

Antiviral Activity of Ocimium Sanctum

(Bawankule et al., 2015**)**
The Phytoactive chemical of Tulsi which is responsible for its antiviral activity is the essential oil Eugenol. Different types of extracts of Ocimum sanctum have anti-viral activity against different viruses. E.g. Hematopoietic Necrosis Virus **(**IHNV**)**, Poliovirus Type 3, herpes virus **(**HSV**)**, hepatitis B virus, New Castle Disease virus. Also, there is evidence that Ocimum sanctum is effective against Swine flu. It can help prevent and also decrease the severity and duration of Swine flu. According to Dr. U.K. Tiwari, an herbal medicine practitioner, the anti-flu property of Tulsi has been discovered quite recently by medical experts across the world. Ocimum sanctum has been successfully used in combatting Japanese Encephalitis and the same theory applies to Swine flu. Tulsi improves the body's overall defense mechanism including its ability to fight viral disease, speed up the recovery process, and also help in strengthening the immune system of the body. Also, extracts of O. sanctum and A. arabica showed significant virucide activity against the H9N2 virus in the Ovo model. **(**Ghoke et al., 2018**)**

Indian medicinal plants and their possible effect on covid-19

(Vellingiri et al., 2020**)**
COVID-19 is a world pandemic that has been recently spread affecting a large population. The virus causing the disease is named SARS-CoV-2. The virus primarily affects the lungs. Being having a viral genome **(**RNA as a genetic constituent**)**, it continuously undergoes mutation, overcomes the defense system of the body **(**Immune system**)**, and multiplies rapidly.

As till now there are no antiviral vaccine or medication has been developed for this deadly disease, hence the only method to keep oneself protected from this virus is to strengthen the body's immune system. Here we focus on Ayurvedic herbal medication for boosting the defense mechanism of the body.

Science ancient times, Indian herbs have been used as a treatment and preventive source for several diseases, including respiratory viral infections.

The main benefit of using these herbs in viral respiratory infection is achieved by building the immune system and stimulating it, and inflammation-modulating effects of managing the immune system. The holistic approach of the AYUSH system of medicine focuses on prevention through lifestyle modification, dietary management, prophylactic interventions for improving immunity, and simple remedies based on a presentation of the symptoms.

Studies on coronavirus using medicinal plants are rather minimal in India, yet certain studies have shown anti-mouse coronaviral activity (a surrogate of SARS CoV) by the plants Indigofera tinctorial, Vitex trifolia, Gymnema Sylvestre, Abutilon Indicum, Leucas Aspera, Cassia alata, Sphaeranthus indicus, Clitoriaternatea, Clerodruminerme Gareth, Pergulariadaemi, and Evolus alsinoides in Tamil Nadu. Among these plants mentioned, e.g. Vitex trifolia and Sphaeranthus indicus have been found to reduce inflammatory cytokines using the NF-KB pathway, a pathway that has been implicated in respiratory distress in SARS-CoV. Similarly, other medicinal plants show their mechanism for building up or stimulating the immune system for fighting out the deadly virus.

Antioxidant Activities of Ocimum Sanctum against Cadmium Induced Toxicity

(N et al., 2017)

Researches show the antioxidant property of Ocimum sanctum hydroalcoholic extract against Cadmium-induced toxicity in albino rats. Oral administration of cadmium as CdCl2 (6.0 mg/kg body weight) led to significant elevation of lipid peroxidation (LPO) levels and significantly decreased Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Reduced Glutathione (GSH) and Vitamin C (Ascorbate) levels.

Administration of Ocimum sanctum extract (100 mg/kg body weight) and (200 mg/kg body weight, PO) before and after cadmium intoxication showed a significant decrease in LPO levels and significant increase in SOD, CAT, GPx, GSH and Ascorbate levels. This suggests that oral administration of Ocimum sanctum extract provides significant protection against cadmium-induced toxicity in Wistar albino rats.

Apart from these above-mentioned properties, various parts of the Tulsi plant are in different extracts that are used for curing several pharmacological abnormalities. E.g. Ocimum sanctum root extracts show anti-inflammatory, analgesic, and antipyretic activity(Asha et al., 2001). Methanolic extract of

Ocimum sanctum leaves shows Anthelmintic activity(Inbaneson et al., 2012). Ethanolic extract of Tulsi has an antiplasmodial effect against Plasmodium falciparum(Prakash & Gupta, 2000). The seed oil of Ocimum sanctum was evaluated for chemopreventive activity against subcutaneously injected 20-methylcholanthrene induced- fibrosarcoma tumors in the thigh region of Swiss albino mice(Prakash & Gupta, 2000).

Extraction Method (Tulsi Leaves)

- Krishna Tulsi leaves (Purple Green colored) were collected from the area between the IFS and IBS department of GFSU. The leaves were thoroughly washed using distilled water and were spread over filter paper for drying. It took around 3-4 hours for the drying.
- Then it was shifted to the basement lab and left for complete drying for 2-3 days. (Not to be dried under direct sunlight which may result in loss of major phytochemical constituent present in the leaves) Since the lab was maintained at a low temperature (20ºC), hence it took a long (around 1 week) for the leaves to dry.
- The dried leaves were now crushed using hand (wearing gloves).
- It was then weighed which was found to be around 72 grams.
- The leaves were taken in a beaker (2-liter capacity) and 1-liter double distilled water was added to it. The beaker was covered using Aluminum foil and kept overnight for extraction at room temperature.
- The next morning using cotton and funnel, the extract was filtered and poured in a 2-liter conical flask. The demonstration of the filtration process is as shown in the figure below:

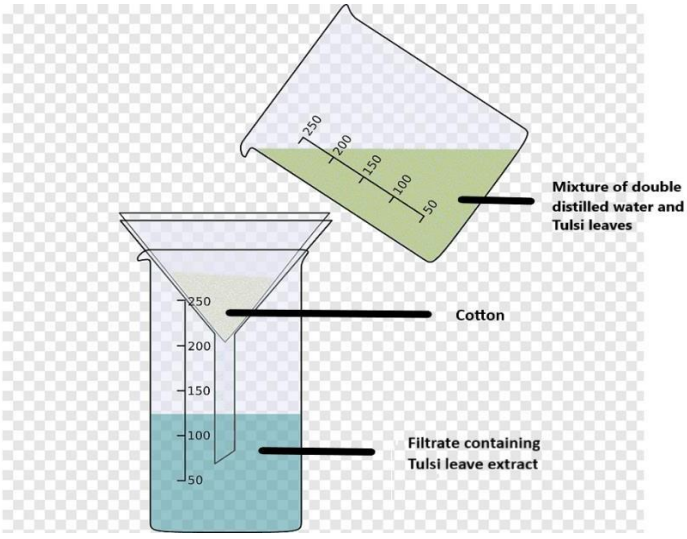


Figure 10: Demonstration of cotton filtration process

- The conical flask was covered using aluminum foil at the neck and stored in a refrigerator.
- The residue **(**leaves**)** were again put back into the beaker and more than 500 ml of double-distilled water was added to it and left overnight again for further extraction.
- The filtrate was obtained again similarly using cotton and funnel in another conical flask which was covered with aluminum foil and stored in the refrigerator.
- The residues were again put back into the beaker and more than 250 ml of double-distilled water was added to it and kept overnight for further extraction.
- The third extract was obtained using a similar method and was stored in the refrigerator.
- The left-over leaf residues were buried in the nearby soil.
- The first extract **(**around 700 ml**)** which was obtained was concentrated in a **Rotary Evaporator** at 75°C by adding a small quantity of sample repeatedly in a 1-liter round bottom flask.

- **(**Pandey et al., 2013**)**

Figure 11: Rotary Evaporator **(**IRD, GFSU**)**

- Finally, a concentrated sample **(**around 30-35 ml**)** was obtained in the RB flask. Now, 600ml of double distilled water was added to the concentrate in the RB flask and was kept overnight. The next day this was again concentrated using a rotary evaporator at 75°C.
- Finally, around 15 ml of thick concentrated extract of Tulsi was obtained and poured in a volumetric flask, and stored in the refrigerator.
- The second extract which was obtained was concentrated using a similar method as above and around 5ml of the concentrated product was obtained and stored in the refrigerator.
- Similarly, the third extract was also concentrated using a rotary evaporator, but the concentrate obtained was very dilute and in a very small quantity.

Rotary Evaporator Conditions/Parameters:

- Required temperature- 75ºC (+/- 5)
- Rpm of the rotary- 70-100
- Vacuum pump must be on
- Chiller must be connected
- RB flask must just touch the water layer

Materials Required

Chemicals

- Calcium chloride was produced from Merck
- Magnesium sulfate, Potassium dihydrogen orthophosphate, Sodium hydrogen orthophosphate, Ammonium chloride, Sodium chloride, Uracil, Bacteriological agar, Peptone, Sodium hydroxide, and Cholesterol were produced from SRL.
- Absolute ethanol was produced from Ureca Consumers co. op. stores.
- Double distilled water and distilled water
- Sodium hypo chloride solution for Axanizing solution preparation

Glasswares and Plasticwares

Conical flask, measuring cylinders, disposable serological pipettes, beakers, glass spreader, glass slide, coverslip, per dish **(**9 mm**)**, falcon tube, 96 well plate, Eppendorf tube, Funnel, Round Bottom Flask **(**RB

flask) [1-liter capacity]

Miscellaneous

Food wrap (cellophane sheet), aluminum foil, spatula, parafilm, needle (worm picker), cotton, micropipette, tissue paper, butter paper, filter paper, micropipette, micropipette tips, dispenser, pair of scissors, distilled water, spirit lamp.

Biological Materials

Caenorhabditis elegans: wild type: N2 strain: BZ- 555 strain Animals were received as a gift from DR. Amir Nazir, Sr. Scientist and Head of Functional Genomics and Molecular Toxicology Laboratory, Division of Toxicology, the council of Scientific and Industrial Research (CSIR) -Central Drug Research Institute (CDRI), Lucknow. Escherichia coli: OP 50 (uracil deficient) strain Krishna Tulsi plant (purple-colored leaves) which was grown in the garden area between IFS and IBS department of GFSU

Instruments

- Rotary Evaporator
- Stereo microscope- Micros Austria: MCX51LED, MC50LED
- BOD incubator- Thermo Scientific
- Water purification system- Lablink Xtrapure Plus
- Digital Autoclave- PSI
- Digital pH meter- LAqua Ph meter by Horiba scientific
- Laminar fume hood with UV light source
- Electronic weighing balance- Rentech
- Orbital Incubator Shake Spire
- Vortex Mixer- Lab net

Protocols

Preparation of Ngm Agar Plates (For 500 Ml)

(Nematode Growth Media)

- The size of conical flask was twice to that the amount of media required.

- Following were weighed and added in the conical flask:

1. NaCl – 1.5 gram
2. Peptone – 1.25 gram
3. Agar – 8.5 gram
4. Distilled water – 487.5 ml

- The above mixture was then autoclaved **(**121ºC for 15 minutes**)**
- Following things are added to it:

1. Freshly prepared Cholesterol – 5 mg/ml in 100% ethanol **(**C_2H_5OH**)**
2. $CaCl_2$ - 1.47 gm in 10ml distilled water **(**1 M solution**)**
3. $MgSO_4$ - 1.2 gram in 10 ml distilled water **(**1 M solution**)**
4. KH_2PO_4 - 3.4 gram in 25 ml distilled water **(**1 M solution, pH should be 6**)**

[0.5 ml each of i, ii, iii, and 12.5 ml of iv was later added to the conical flask]

- Except for Cholesterol, the rest of all solutions are needed to be autoclaved.
- The fuming hood was decontaminated using UV light and then the methanol lamp was lit.
- After autoclave, they were taken out and kept in the fume hood.
- Meanwhile, fresh plates were taken out and kept in the hood and marked with a date.
- Using a micropipette and tips, the required amount of solutions is added to the conical flask and mixed properly.
- Lids of the fresh plates were opened and using a serological pipette **(**25 ml**)** fitted in a dispenser **(**pipette controller**)**, 18-22 ml of agar solution was poured into each plate.
- The plates were allowed to cool for an hour, after which the lids were closed and a stock of 5 was made and wrapped into cellophane sheets followed by aluminum foil and was kept in the fridge.

Preparation of M Buffer Solution

- Reagents required for 1 Liter:
    1. Distilled water – 600ml
    2. KH_2PO_4 - 3g
    3. Na_2HPO_4 - 6g

4. NaCl – 5g
5. $MgSO_4$ **(**1 M**)** - 1ml **(**0.12 g in 1 ml**)**

- Above reagents are mixed well and made up to 1 liter **(**After adding each, add 100ml of distilled water each time to ensure even mixing and finally made up to 1 liter**)**.
- Transfer 500 ml of it to another reagent bottle and autoclave both, labeled with name and date on it.
- Cool to room temperature and store it in the fridge.

Preparation of Minimum Essential Media (mem) for E. Coli

- 60 ml of M9 was taken in a sterile measuring cylinder and was autoclaved.
- To this following are added:
    1. 1.5 mL of OP 50 culture
    2. 0.75 mL/750 µL of NH_4Cl
    3. 0.75 mL Glucose **(**20% in distilled water
    4. 15 µL of Uracil **(**0.2 % in autoclaved distilled water**)**.

- Volume was made up to 75 ml by adding more M9, and to avoid contamination, all the mixing are carried out in a fume hood.
- The final solution was transferred in a sterile conical flask and closed with a cotton plug and was kept in an incubator shaker at 37ºC overnight till turbidity was observed.
- The next day, if sufficient turbidity is not found then again 15 µL of Uracil, can be added and kept in an incubator shaker for more than 2-3 hours at 37ºC.
- The culture is stored in BOD.

Seeding of Plates

- Requirements:
    1. Fresh NGM plates
    2. MEM with E. Coli
    3. Spreader
    4. Micropipette with tips
    5. Methanol fueled lamps
- The laminar hood is sterilized
- Plates are taken out from the fridge and kept at room temperature
- Meanwhile, the incubator is turned on and set at 37ºC to attain require temperature

- Plates are now placed in the incubator in the up and down manner **(**30 minutes each manner**)** and then the plates are placed in the fume hood
- Methanol lamp is lit and E. Coli is poured **(**0.5 ml or 500 µl**)** onto the NGM plates with the help of a micropipette
- It is then spread on the plates using a sterilized spreader **(**with continuous cooling and heating**)**
- Plates are then dried at room temperature and then labeled
- The lids are closed and placed in the incubator overnight **(**12-18 hours**)** without sealing
- The next day, the plates are taken out from the incubator and placed in a fume hood
- If chunking is required, the plates are chunked with respective strain
- If not required, then the plates are sealed using parafilm, made to the stock of 5, and wrapped with aluminum foil
- The stock is placed in the fridge for further use

Culturing of Worms

To perform culturing, the worms are needed to be transferred from a well-populated plate of worms to a fresh seeded plate. For transferring worms, a process called **"chunking"** is done, i.e., a chunk of agar from a well-populated plate of worms are taken using a sterile spatula and placed inverted on a fresh seeded plate so that worms could crawl out of chunk and spread on the bacterial lawn of the new plate.

Chunking involves the following steps:

- Freshly seeded plates are taken out from the fridge and placed at room temperature in the hood
- Meanwhile, the incubator is turned on **(**to attain a temperature of 37ºC**)**
- Plates were now placed in the incubator in an up and down manner for 1 hour **(**30 minutes in each manner**)**
- Chunk plates of required strain are taken out from BOD and observed under a microscope for live animals and the portion is marked and placed in a fume hood
- Fresh seeded placed are taken out of the incubator and placed in a fume hood
- Methanol lamp is lit and the flat portion of a spatula is heated on it and allowed to cool
- The marked portion of the worm plate is cut and the chunk is placed in a downward direction on a freshly seeded plate
- The lid is now closed, sealed with parafilm, and placed in BOD

Transferring a single worm was done by a worm -picking. A worm-picker is made by flattening and folding the tip of a needle or syringe using a pestle to make it blunt, as sharp edges may damage the worms and poke a hole in the plate. To avoid contamination, the picker was sterilized by flaming. For picking worms, the worms are identified under a stereomicroscope and swiped off the plate with the picker, and transferred on a fresh plate by gently placing the picker on a fresh plate.

Age Synchronization

To perform any experiment on the model organism **(**C. elegans here**)**, the worms on which any kind of exposure is done must be in the same stage of their lifecycle. The process of achieving the experimental worms into the same stage is known as **Age synchronization**. This can be done by collecting only the eggs of the worm and allow them to hatch together so that larva belonging to a single stage can be obtained.

It involves the following steps:

- The fume hood is sterilized using UV light **(**15 minutes UV followed by 15 minutes tube light**)**
- A chunked plate is taken out of BOD and looked under a microscope for the presence of adult worms and eggs.
- Meanwhile, the M9 buffer solution is taken out of the fridge and kept in the hood to bring it to room temperature.
- One 15ml falcon tube and a 2ml centrifuge tube are taken out and kept in the hood.
- Now, the Axanizing solution is prepared in the 2ml centrifuge tube by adding 0.5ml of 5N NaOH and 1ml Sodium hypo chloride solution.
- Using 1ml M9 buffer, the selected chunked plate is washed using a micropipette **(**worm washing**)**, and the wash is collected in a 15ml falcon tube. NOTE- Before collecting the worm wash, cut the tip from the end so that the maximum number of C. elegans can be collected. M9 buffer wash is repeated for 3 times.
- Now the prepared 1.5ml of Axanizing solution is added to it and the bottom of the falcon tube is tapered continuously for 7-8 minutes with maximum possible speed using fingers.
- Then immediately centrifuge it at 3410 pm, at 22ºC for 30 seconds.
- The supernatant is now discarded using the micropipette, and then a little if M9 buffer is again added to it and tap for more than 2 minutes as the above-mentioned manner.
- Centrifuge it again at 3410 pm, at 22ºC for 30 seconds.
- The supernatant is again removed using a micropipette, leaving behind a little **(**around 0.5 ml**)** of the solution.

- Dissolve the obtained precipitate **(**eggs**)** onto the solution and pour it on a fresh unseeded NGM plate. [Unseeded plates are used to cease the larva in the L1 stage-if no food is available on the plate, then the worms will not grow to the further stage]
- This plate is now sealed with parafilm and kept in BOD at 22ºC.
- After 8-12 hours, the plates are observed under a microscope for the presence of L1 larva **(**Age synchronized**)**.

Note

- While washing and picking up C. elegans, the tips are needed to be cut. This provides greater space and more no. of worms can be picked up.
- During centrifuge, bottle type arrangement is needed to be set, and balancing of the bottles is done using another falcon tube containing an approximately equal quantity of distilled water as that of our solution.
- After tapping for 7-8 minutes, an immediate centrifuge is required, therefore this step is done as quickly as possible.

Result and Discussion

Preparation of Ocimum sanctum crude extract

According to the referred research article, they collected Arial parts of *O. sanctum* from the experimental fields where here, we collected Krishna Tulsi leaves from the herbs grown in the garden area within the university. Cleaning and washing were done using distilled water. The drying process was made by the researchers by applying the conventional method of spreading the leaves on the ground under a shade, while in the method developed here, we used large filter papers on which the washed leaves were spread for drying purposes. Unlike the conventional method of drying on the ground under a shade, the leaves were kept on filter papers in an air-conditioned laboratory whose temperature was maintained around 22ºC. Hence, there was no direct or indirect exposure of the leaves to heat or sunlight. It took around a week or two for the leaves to get dried completely. The dried leave material was now crushed into coarse powder and further used for the preparation of the extract. The approximate weight of the obtained coarse powder was measured using a conventional weighing balance and was noted down.

The dried powder was dipped overnight in double-distilled water in an Erlenmeyer flask. In the research article, 100g of powder was obtained onto which 200ml of double distilled water was added. While the weight of the dried powder we obtained was around 72g to which 1- liter double distilled water was

added to ensure that the dried stuff is completely dipped into the water level. The container was covered using aluminum foil and left as it is overnight for extraction **(**at room temperature**)**.

The next day, the mixture of water and *O. sanctum* dried powder was filtered through cotton **(**using a funnel**)**, and the filtrate was collected in a 1-liter conical flask **(**about 700 ml was collected**)**. This was the *first extract* that was obtained and was stored in the refrigerator. To the left-over residue, another 500ml of double distilled water was added and covered with aluminum foil, and again left overnight for the next round of extraction. The next day, the cotton filtration process was again repeated similarly and the *second extract* was obtained. The residue was again added with 250ml of double distilled water and left overnight. *The third extract* was then obtained, though it was very light in color due to the little amount of phytochemical left in the residue.

The *first extract* which was around 700ml was now concentrated using ***a Rotary evaporator***. Initially, a little quantity of extract was taken in the RB flask and was fit to the rotor. The chiller and vacuum pump motor were switched on. The temperature was set to 75ºC with an rmp of 70 which was gradually increased to 100. This temperature was obtained after a lot of trial and error **(**This resulted in a loss of a little quantity of solution due to splashing**)**. Also, the rmp was ramped up and down within the range many times. Once, the solution started to evaporate, and the method was set, then small quantities of the solution were added repeatedly and a thick concentration of Tulsi was obtained in the RB flask. To this again 600ml of double distilled water was added and left overnight. The next day, this was again concentrated in the similar method and rotary parameters. Finally, a thick concentrated crude extract **(**15ml**)** of *Ocimum sanctum* was obtained. This was then poured into a volumetric flask and stored in the refrigerator. Similarly, the second and third extract were also concentrated.

In the referred research article, the concentrated filtrate which was obtained was poured and evaporated on particulates. The dried residue of the extract was used to prepare a stock solution in water. The drying process can also be carried out by a process known as **lyophilization** or **cryodesiccation**, also known as freeze-drying. **Freeze drying** is a low-temperature dehydration process that involves freezing the product, lowering pressure, then removing the ice by sublimation. This process may preserve the essential phytochemicals in the extract unlike the conventional method of evaporation which may result in loss of active constituents.

Preparation of Nematode Growth Media (NGM) agar plates

Chemicals used for NGM preparation i.e. NaCl, Peptone, Agar, Cholesterol, $CaCl_2$, $MgSO_4$ are almost the same as those mentioned in the standard worm book. Also, their required quantities are the same as that of the standard. It is only the KP buffer stock that makes the difference.

KP buffer stock includes K_2HPO_4 **(**5g**)** and KH_2PO_4 **(**30g**)** whose pH is maintained as 6. This is as per the standard protocol. But here we use only KH_2PO_4 **(**3.4 gm in 25ml distilled water**)**, with pH maintained as 6 as a buffer solution. While preparing this, firstly, 3.4gm of KH_2PO_4 was weighed and taken in a 25ml falcon tube. To this 17.5ml of distilled water was added and vortexed until a clear transparent solution is obtained. To this now, 5N NaOH is added dropwise using a micropipette. After adding each drop of NaOH, the solution is vortexed and the pH of the solution is measured using a pH meter. Accuracy of pH 6 is tried to attain. Once pH 6 is attained, the volume is now made up to 25ml using distilled water and the buffer is now ready to use after autoclave. Except for Cholesterol, remaining all solutions are needed to be autoclaved. Finally, the solutions are added in their required quantities as mentioned in the method and the NGM agar plates are prepared and used for worm culturing and other purposes.

Age synchronization of the worms

In the referred research article, for synchronizing the worm population, wild type *C. elegans* strain was given hypochlorite treatment, and the resulting egg was placed on NGM plates spotted with E. coli OP50 bacteria. All worms were grown in a temperature-controlled incubator. Different pharmacological doses of *O. sanctum* crude extract were added directly to the OP50 food source to feed the worms.

Then the required assay was carried out. Quite a similar method was used here for the age synchronization process which involved the use of an Axanizing solution which is a mixture of 0.5ml 5N NaOH and 1ml Sodium hypochlorite solution. Sodium hypochlorite **(**NaOCl**)** commonly known as liquid bleach or simply bleach, is a household chemical widely used as a disinfectant or a bleaching agent. NaOCl is unstable and easily decomposes in basic media to liberate chlorine which results in bleaching property. The amazing solution does the same task i.e. when NaOH is added to NaCl, chlorine is liberated and it removes all the soft tissues of the worms exposing the eggs. So, when the Axanizing solution is added to the M9 worm wash and the falcon tube is tapered continuously, then the soft tissues of the worm get dissolved in the solution exposing the eggs outside.

When the solution is immediately centrifuged **(**at 3410 rpm for 30 seconds**)**, then the eggs settle down and the debris remains in the supernatant solution which is then removed using a micropipette.

Unlike the one mentioned in the research article, the obtained eggs were poured on an unseeded plate. It is because when the worms hatch out of the eggs, they be in their larva 1 **(**L1**)** stage and any kind of exposure can be done. After pouring the eggs, the plates are kept in BOD **(**22ºC**)**. L1 staged worms can be seen on the plates after 8-12 hours.

Conclusion

The ever-increasing rise of debilitating chronic diseases and stress in the population has enforced the necessity for searching for novel therapeutics. The study regarding the medicinal properties of the Tulsi plant **(***Ocimum sanctum***)** has provided several health benefits of Tulsi including anti-oxidant, anti-aging, promoting healthy heart, treating kidney stone, relieves headaches, fights acne, relieves fever, improves eye health, cures respiratory disorders, etc. The extract also has been proved to be a strong free radical and increase resistance against thermal stress. It is also suggested that its antioxidant capacity results in a protective and life span extending action. Phytochemical constituents of Tulsi modulates several signaling pathways and increases stress tolerance and life span in *C. elegans.*

References

1.Asha, M. K., Prashanth, D., Murali, B., Padmaja, R., & Amit, A. (2001). Anthelmintic activity of essential oil of Ocimum sanctum and eugenol. Fitoterapia, 72(6), 669–670. https://doi.org/10.1016/S0367-326X(01)00270-2

2.Bawankule, D., Kumar, A., Agarwal, K., Maurya, A., Shanker, K., Bushra, U., & Tandon, S. (2015). Pharmacological and phytochemical evaluation of Ocimum sanctum root extracts for its antiinflammatory, analgesic and antipyretic activities. Pharmacognosy Magazine, 11(42), 217. https://doi.org/10.4103/0973-1296.157743

3.Bhat, S. S., Kochikar Pai, R., Salman, A., & Chandra, J. (2015). Use of an Extract of Indian Sacred Plant Ocimum sanctum as an Anticariogenic Agent: An in vitro Study. International Journal of Clinical Pediatric Dentistry, 8(2), 99–101. https://doi.org/10.5005/jp-journals-10005-1292

4.Bhattacharya, S. (2018). Medicinal plants and natural products can play a significant role in mitigation of mercury toxicity. Interdisciplinary Toxicology, 11(4), 247–254. https://doi.org/10.2478/intox-2018-0024

5.Bhattacharyya, P., & Bishayee, A. (2013). Ocimum sanctum Linn. (Tulsi): An ethnomedicinal plant for the prevention and treatment of cancer. Anti-Cancer Drugs, 24(7), 659–666. https://doi.org/10.1097/CAD.0b013e328361aca1

6.Cohen, M. M. (2014). Tulsi - Ocimum sanctum: A herb for all reasons. Journal of Ayurveda and Integrative Medicine, 5(4), 251–259. https://doi.org/10.4103/0975- 9476.146554

7.Fathiazad, F., Matlobi, A., Khorrami, A., Hamedeyazdan, S., Soraya, H., Hammami, M., Maleki-Dizaji, N., & Garjani, A. (2012). Phytochemical screening and evaluation of cardioprotective activity of ethanolic extract of Ocimum basilicum L. (basil) against isoproterenol induced myocardial infarction in rats. DARU, Journal of Pharmaceutical Sciences, 20(1), 1–10. https://doi.org/10.1186/2008-2231-20-87

8.Gautam, M. K., & Goel, R. K. (2014). Toxicological study of Ocimum sanctum Linn leaves: Hematological, biochemical, and histopathological studies. Journal of Toxicology, 2014. https://doi.org/10.1155/2014/135654

9.Ghoke, S. S., Sood, R., Kumar, N., Pateriya, A. K., Bhatia, S., Mishra, A., Dixit, R., Singh, V. K., Desai, D. N., Kulkarni, D. D., Dimri, U., & Singh, V. P. (2018). Evaluation of antiviral activity of Ocimum sanctum and Acacia arabica leaves extracts against H9N2 virus using embryonated chicken egg model. BMC Complementary and Alternative Medicine, 18(1), 1–10. https://doi.org/10.1186/s12906-018-2238-1

10.Guest, J., & Grant, R. (2016). The Benefits of Natural Products for Neurodegenerative Diseases. Advances in Neurobiology, 12, 199–228. https://doi.org/10.1007/978-3-319- 28383-8

11.Hening, P., Mataram, M. B. A., Wijayanti, N., Kusindarta, D. L., & Wihadmadyatami, H. (2018). The neuroprotective effect of Ocimum sanctum Linn. ethanolic extract on human embryonic kidney-293 cells as in vitro model of neurodegenerative disease. Veterinary World, 11(9), 1237–1243. https://doi.org/10.14202/vetworld.2018.1237-1243

12.Inbaneson, S. J., Sundaram, R., & Suganthi, P. (2012). In vitro antiplasmodial effect of ethanolic extracts of traditional medicinal plant Ocimum species against Plasmodium falciparum. Asian Pacific Journal of Tropical Medicine, 5(2), 103–106. https://doi.org/10.1016/S1995-7645(12)60004-2

13.Jamshidi, N., & Cohen, M. M. (2017). The Clinical Efficacy and Safety of Tulsi in Humans: A Systematic Review of the Literature. Evidence-Based Complementary and Alternative Medicine, 2017. https://doi.org/10.1155/2017/9217567

14.Jürges, G., Sahi, V., Rodriguez, D. R., Reich, E., Bhamra, S., Howard, C., Slater, A., & Nick, P. (2018). Product authenticity versus globalisation—The Tulsi case. PLoS ONE, 13(11), 1–22. https://doi.org/10.1371/journal.pone.0207763

15.Kusindarta, D. L., Wihadmadyatami, H., & Haryanto, A. (2016). Ocimum sanctum Linn. stimulate the expression of choline acetyltransferase on the human cerebral microvascular endothelial cells. Veterinary World, 9(12), 1348–1354. https://doi.org/10.14202/vetworld.2016.1348-1354

16.Lahon, K., & Das, S. (2011). Hepatoprotective activity of Ocimum sanctum alcoholic leaf extract against paracetamol-induced liver damage in Albino rats. Pharmacognosy Research, 3(1), 13–18. https://doi.org/10.4103/0974-8490.79110

17.Mukherjee, P. K., Harwansh, R. K., Bahadur, S., Banerjee, S., Kar, A., Chanda, J., Biswas, S., Ahmmed, S. M., & Katiyar, C. K. (2017). Development of Ayurveda – Tradition to trend. Journal of Ethnopharmacology, 197, 10–24. https://doi.org/10.1016/j.jep.2016.09.024

18.N, B., A, A., M, T., GM, K., & MT, A. (2017). Pharmacological Evaluation of Ocimum sanctum. Journal of Bioequivalence & Bioavailability, 09(03), 387–392. https://doi.org/10.4172/jbb.1000330

19.Nangia-Makker, P., Tait, L., Shekhar, M. P. V., Palomino, E., Hogan, V., Piechocki, M. P., Funasaka, T., & Raz, A. (2007). Inhibition of breast tumor growth and angiogenesis by a medicinal herb: Ocimum gratissimum. International Journal of Cancer, 121(4), 884–894. https://doi.org/10.1002/ijc.22733

20.Ononamadu, C. J., Alhassan, A. J., Imam, A. A., Ibrahim, A., Ihegboro, G. O., Owolarafe, A. T., & Sule, M. S. (2019). In vitro and in vivo anti-diabetic and anti- oxidant activities of methanolic leaf extracts of Ocimum canum. Caspian Journal of Internal Medicine, 10(2), 162–175. https://doi.org/10.22088/cjim.10.2.162

21.Organism, A. S. A. G. (2014). C elegans anatomy and lifecycle.

22.Pandey, R., Gupta, S., Shukla, V., Tandon, S., & Shukla, V. (2013). "Antiaging, antistress and ROS scavenging activity of crude extract of ocimum sanctum (L.) in caenorhabditis elegans (Maupas, 1900)". Indian Journal of Experimental Biology, 51(7), 515–521.

23.Parasuraman, S., Thing, G. S., & Dhanaraj, S. A. (2014). Polyherbal formulation: Concept of ayurveda. Pharmacognosy Reviews, 8(16), 73–80. https://doi.org/10.4103/0973-7847.134229

24.Prakash, J., & Gupta, S. K. (2000). Chemopreventive activity of Ocimum sanctum seed oil. Journal of Ethnopharmacology, 72(1–2), 29–34. https://doi.org/10.1016/S0378- 8741(00)00194-X

25.Ramesh, B., & Satakopan, V. N. (2010). Antioxidant activities of hydroalcoholic extract of ocimum sanctum against cadmium induced toxicity in rats. Indian Journal of Clinical Biochemistry, 25(3), 307–310. https://doi.org/10.1007/s12291-010-0039-5

26.Rastogi, S., Kalra, A., Gupta, V., Khan, F., Lal, R. K., Tripathi, A. K., Parameswaran, S., Gopalakrishnan, C., Ramaswamy, G., & Shasany, A. K. (2015). Unravelling the genome of Holy basil: An "incomparable" "elixir of life" of traditional Indian medicine. BMC Genomics, 16(1). https://doi.org/10.1186/s12864-015-1640-z

27.Rupani, R., & Chavez, A. (2018). Medicinal plants with traditional use: Ethnobotany in the Indian subcontinent. Clinics in Dermatology, 36(3), 306–309. https://doi.org/10.1016/j.clindermatol.2018.03.005

28.Sethi, J., Yadav, M., Sood, S., Dahiya, K., & Singh, V. (2010). Effect of tulsi (Ocimum Sanctum Linn.) on sperm count and reproductive hormones in male albino rabbits. International Journal of Ayurveda Research, 1(4), 208. https://doi.org/10.4103/0974- 7788.76782

29.Shetty, S., Udupa, S., & Udupa, L. (2008). Evaluation of antioxidant and wound healing effects of alcoholic and aqueous extract of Ocimum sanctum Linn in rats. Evidence- Based Complementary and Alternative Medicine, 5(1), 95–101. https://doi.org/10.1093/ecam/nem004

30.Shimizu, T., Torres, M. P., Chakraborty, S., Souchek, J. J., Rachagani, S., Kaur, S., Macha, M., Ganti, A. K., Hauke, R. J., & Batra, S. K. (2013). Holy Basil leaf extract decreases tumorigenicity and metastasis of aggressive human pancreatic cancer cells in vitro and in vivo: Potential role in therapy. Cancer Letters, 336(2), 270–280. https://doi.org/10.1016/j.canlet.2013.03.017

31.Upadhyay, A. K., Chacko, A. R., Gandhimathi, A., Ghosh, P., Harini, K., Joseph, A. P., Joshi, A. G., Karpe, S. D., Kaushik, S., Kuravadi, N., Lingu, C. S., Mahita, J., Malarini, R., Malhotra, S., Malini, M., Mathew, O. K., Mutt, E., Naika, M., Nitish, S., … Sowdhamini, R. (2015). Genome sequencing of herb Tulsi (Ocimum tenuiflorum) unravels key genes behind its strong medicinal properties. BMC Plant Biology, 15(1), 1– 20. https://doi.org/10.1186/s12870-015-0562-x

32.Vellingiri, B., Jayaramayya, K., Iyer, M., Narayanasamy, A., Govindasamy, V., Giridharan, B., Ganesan, S., Venugopal, A., Venkatesan, D., Ganesan, H., Rajagopalan, K., Rahman, P. K. S. M., Cho, S. G., Kumar, N. S., & Subramaniam, M. D. (2020). COVID-19: A promising cure for the global panic. Science of the Total Environment, 725, 138277. https://doi.org/10.1016/j.scitotenv.2020.138277

33.Verma, S. (2016). Chemical constituents and pharmacological action of Ocimum sanctum (Indian holy basil-Tulsi). The Journal of Phytopharmacology, 5(5), 205–207. www.phytopharmajournal.com

34.Yamani, H. A., Pang, E. C., Mantri, N., & Deighton, M. A. (2016). Antimicrobial activity of Tulsi (Ocimum tenuiflorum) essential oil and their major constituents against three species of bacteria. Frontiers in Microbiology, 7(MAY), 1–10. https://doi.org/10.3389/fmicb.2016.00681

35.www.sciencedirect.com, www.ncbi.nlm.nih.gov/books, www.wikkipedia.com

Volume 2 Issue 2 March 2021