A COMPREHENSIVE REVIEW ON PHYTOCHEMICAL EVALUATION AND PHARMACOLOGICAL ACTIVITIES OF OCIMUM SANCTUM

A project report submitted in partial fulfilment for the award of the degree of

MASTER OF SCIENCE (M.Sc.) in CHEMISTRY

Submitted by

&

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DECLARATION

We, Sakshi and Sarika Yadav, hereby certify that the work which is being submitted in this major project report entitled "A Comprehensive review on Phytochemical evaluation and Pharmacological activities of *Ocimum sanctum*" in the partial fulfilment for the award of the degree of Master of Science at Delhi Technological University is an authentic record of our own work carried out by us under the supervision of Dr. Richa Srivastava (Assistant Professor, Department of Applied Chemistry, Delhi Technological University). We, further declare that the project report has not been submitted to any Institute/ University for the award of any degree or diploma or any other purpose whatsoever. Also, it has not been directly copied from any source without giving its proper reference.

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CERTIFICATE

I hereby certify that the project Dissertation titled "A comprehensive review on phytochemical evaluation and pharmacological activities of *Ocimum sanctum*." which is submitted by Sakshi, Sarika 2K21/MSCCHE/40 and 2K21/MSCCHE/42, Department of applied chemistry, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of degree of Master of Science, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not be submitted in the part of full for any degree or diploma to this university or elsewhere.

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Sakshi & Sarika yadav

ABSTRACT

Tulsi is often regarded as "the incomparable one", "Elixir of life" and Queen of herbs". Tulsi is being used for about a thousand years to support a healthy mind, body, and spirit. Tulsi is believed to have originated in northern central India but grows throughout the Indian subcontinent, Nepal, Bangladesh and much of Southeast Asia. Across different cultures, it is a symbol of purity. It is used to treat various types of diseases and disorders from ancient times. It has tremendous medicinal properties and is used to treat heart diseases, digestive issues, respiratory problems, menstrual cramps, skin related disorders like acne, and mouth ulcers and even helps in controlling blood sugar levels. It is significantly used as an immune-modulatory, antihypertensive, antioxidant, antipyretic, antimicrobial and for anticancer activity. Phytochemicals like Eugenol, methyl eugenol, anthocyanins, linalool, ursolic acid are accountable for the pharmacological properties of the Tulsi. In this report we will discuss about the different varieties of Tulsi, its classification, traditional uses, it's phytochemicals constitution and the pharmacological activities

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CHAPTER 1

INTRODUCTION

Tulsi is a sacred plant which has spiritual and therapeutic values to the Hindu community in India [1-2]. The word tulsi is derived from the Sanskrit language which means "The incomparable one" [3]. Tulsi has been used for quite a long time in ancient ayurveda for its ample healing properties. Tulsi extracts are used in treating coughs, common cold, heart diseases [4]. Tulsi is grown all over the country from courtyards in the houses to the gardens in the temples. It is also used commercially in cosmetics and pharmacological industries [5]. The roots and stems of the tulsi plant were used for the treatment of malaria, mosquito and snake bites in ancient times [6-7]. It is an erect, annual plant which can attain a height of 30-60cm at maturity. The leaves have a distinct aroma and are simple, elliptic, oblong, opposite with sub serrate or entire or dentate margins. The flowers are tiny, purple in colour with elongated racemes in close whorls [8]. The important chemical components of tulsi are: oleanolic acid, rosmarinic acid, ursolic acid, eugenol, linalool, carvacrol, β -caryophyllene, β -elemene, and germacrene D. β -elemene shows anticancer properties but its human clinical trials are yet to be made [9].

1.1 TAXONOMIC CLASSIFICATION

It is impossible to study each characteristic of every organism. So certain ways have been devised to make this process easier. All organisms are characterized based on easily observable characters into certain groups called taxa and the process of classification is called as Taxonomy. Taxonomic studies of different organisms have led to the development of common categories such as Kingdom, Division, Class, Order, Family, Genus, and Species and may include different subcategories.

Kingdom	Plantae
Subkingdom	Tracheobionta

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Ignie I I	Indicating th	1e categories to	which	111101	helong
	multicating ti	ne categories to		i uisi	DCIUII25.

Super division:	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae
Genus:	Ocimum

It belongs to the family Lamiaceae and the genus Ocimum has approximately 30 species. [10-12]

1.2 VARITIES OF TULSI

Different types of Tulsi are cultivated all over the world and is mainly categorized into 2 groups. Holy basil-According to ancient ayurveda they are further sub categorized into mainly four types -O. sanctum (Rama-tulsi),O. tenuiflorum (Krishna-tulsi),O. tenuiflorum (Amrita-tulsi) ,O. gratissimum (Vana-tulsi) .Rama and Shyam are the most common ones and together they are classified as O. tenuiflorum where Vana tulsi which is less common is classified as O. gratissimum[13]



Figure 1.1 Rama, Shyam and Vana Tulsi grown in Bangalore, India (pictures by S.K. Bhamra) [13]

Mediterranean Basil-It is the most consumed herb and is found all over the world. It is further sub categorised into seven types sweet basil (O. basilicum),Thai basil (O. thyrsiflora), Purple basil (O. basilicum),Lemon basil (O. citriodorum),Vietnamese basil (O. cinnamon),American basil (O. americanum),African blue basil (O. kilimandscharicum) ,Italian genovese basil (O. basilicum)[14]

1.3 GEOGRAPHICAL LOCATION

Tulsi is cultivated in tropical and warm regions. It is distributed widely in the areas of Asia, Mantase Islands, and Mediterranean countries. It is also located in Australia, Malaysia, West Africa and some Arab countries. In India, it is grown throughout the country from the Himalayas to the Andaman and Nicobar Islands.[10] It is sacred to the Hindus and is grown near temples. It is an annual herb with a simple, opposite, elliptical, and oblong leaf.[15] It is propagated by the seeds.[16]

2 TRADITIONAL USES OF OCIMUM SANCTUM

PLANT PART	TRADITIONAL USE
Juices of leaves	reduce the effect of snake bite and poisonous
	insects
Dry root with water	helpful for stomach disorders, fever, vomiting
	[17]
Leaves grounded with Hibiscus rosa-sinensis	to treat asthma
flowers	
leaf powdered with cow or goat's milk	to treat diabetes [18]
Boiled Leaves and stems	antiasthmatic, antispasmodic, analgesic,
	antidiarrhoeic, anti-inflammatory
Plant juice	memory enhancer, cardiac tonic [19]
Leaves	burning sensation of feet, palm, and redness of
	eyes

Table 2.2 showing traditional uses of Ocimum sactum

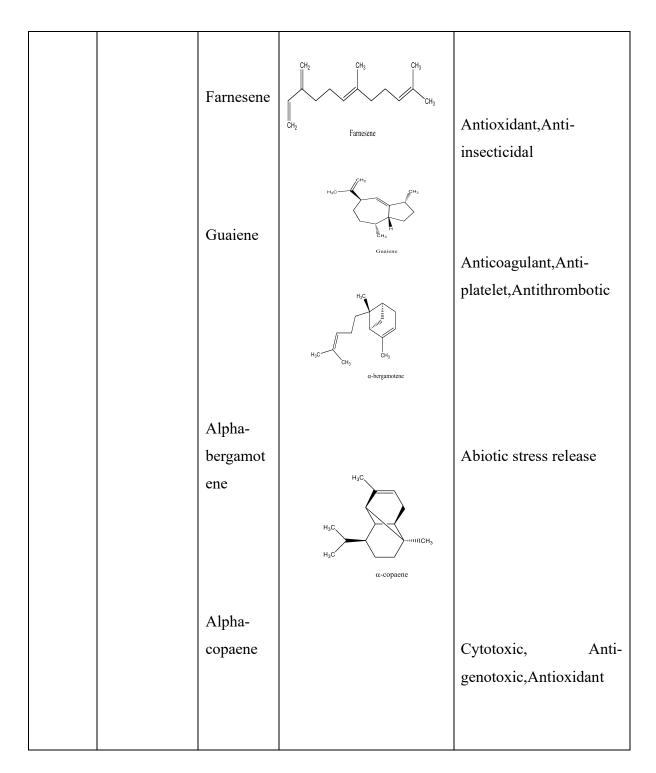
Macerated leaves with camphor	maintain pregnancy
Leaves	typhoid fever [20]
Crushed Leaves	ear boils [21]

3 CHEMICAL CONSTITUENTS

S.NO	CLASS	CHEMI	STRUCTURE	PROPERTIES
		CAL		
		CONSTI		
		TUENT		
1	Flavonoid	Cirsilenol		Antioxidant,Anti
		Cirsimarit		^{or} inflammatory
		in	H ₃ CO H O R ₃	
		Isothymo	R2=OH,R2=H,R3=H;Isothymusin	
		nin	$\begin{split} R_1 = &OH, R_2 = CH_3, R_3 = OH; Isothymunun\\ R_1 = R_2 = R_3 = H; Cirsimaritin\\ R_1 = R_2 = H, R_3 = OCH_3; Cirsilineol \end{split}$	
		Isothymu	K1-K2-11,K3-00113,Citistinicol	
		sin		
2	Sesquiterpen	Alpha-		Analgesic, Antibiotic, Ant
	e	Bisabolol	α-bisabolol	icancer
3	Phenyl	Eugenol	H ₃ CO	Neuro-protective,Local
	propanoid		но	anasthetic,Anti -
			Eugenol	inflammatory,Anti -
				cancer
		Chicoric	HO O O O O O O O O O O O O O O O O O O	
		acid	Cancorre acia	

Table 3.3 showing different chemical constituents

			Antioxidant,Immunosti
			mulatory
Phenolic acid	Rosmarin		Antioxidant, Anti-
	ic acid		inflammatory
		Rosmarínic acid	
Monoterpene		CH.	Antioxidant, Antibacteral
	acetate	H _a c CH _a Menthyl acetate	
	Limonene	H ₃ C Limonene	Anti inflammatory,Motor relaxant
Phenyl	Estragole		Neuron excitability
Propene		H ₃ C O CH ₂	
		Estragole	
	Methyl	H ₂ C OCH ₃	
	Eugenol	Methyl Eugenol	Anticonvulsant,Anasthet ic
Monoterpene alcohol	Linalool	H ₃ C CH ₂	Antihyperalgesic,Antino ciceptive
		H ₃ C CH ₃	
e Sesquiterpen e	Beta- elemene	H_2C H_2C H_2C H_3 CH_3 CH_2 CH_2 CH_2 CH_2 CH_2 CH_2	Anticancer,Antineoplasti c
	Monoterpene Phenyl Propene Propene Monoterpene Sesquiterpen	ic acid ic acid Monoterpene A Benyl Propene Propene Bropene A Bestagole A Best	ic acid $\underset{i \in cid}{\underset{i \in cid}{\atop cid}{\atop}}}}}}}}}}}}}}}}}}}}}}}}}}}}} } i ll i ll}}}}}}}}$



Phyto is derived from the Greek word which means plants. Phytochemicals are the chemical compounds which are present naturally in the plant parts and are used to prevent or cure any disease. More than 5000 phytochemicals have been discovered and yet a large number of them are still to be discovered.[22].The chemical composition of the plant is quite complex and proportion of the active chemical compounds differs in the different species of the genus.[23].Tulsi essential oil has major concentrations of eugenol (more than70%) which is

responsible for the anticancer ,antioxidant and antimicrobial properties.[24,25].Various chemical constituents containing biologically active compound like 70% eugenol (volatile oil), 20% eugenol ,methyl ether, caryophyllene, oleanolic acid, 3% carvacrol, caffeic acid, ursolic acid (triterpenoid), apigenin, luteolin, rosmarinic acid (phenylpropanoid) and chlorophyll have been isolated from the leaves.[26,27]. The O. sanctum leaves also contains cirsilineol, cirsimartin, isothymusin, isothymonin, apigenin, rosmarinic acid [28]. The leaves are rich in phenolics, terpenoids, volatile oils and fatty acid derivative. The seeds contain mucilage, polysaccharide and the seed oil have fatty acids like stearic acid, palmitic acid, oleic acid, linoleic acid where linoleic acid is present in the major amount.[29]. Trace minerals like Zinc, Iron, Manganese are present. Vitamin A and Vitamin C are present along with macro minerals like sodium, Calcium, phosphorous etc[30]. The plants also contain saponin, alkaloids, tannins, glycosides, and traces of citric acid, tartaric acid, and maleic acid. The disparity in the phytochemicals may be due to the geographical or edaphic factors [31]. In Ocimum sanctum the chemical constituents like, methyl eugenol, Beta-elemene, methyl chavicol, linalool, (E)caryophyllene Beta-caryophyllene[32], Aromadendrene oxide, Benzaldehyde, Selinene, Phytol Oleic acid, Sabinene, Aesculectin[33], Ursolic acid, Carvacrol(5-isopropyl-2methylphenol),Estragol(1-allyl-4-methoxybenzene),Phenolic compounds(Cirsilineol,circimaritin, apigenin isothymusin, Rosamarinic acid)[34] 1,8-cineole,

limonene, Bornyl acetate, chicoric acid ,Guaine etc[35] are present.

3.1 PHENOLICS-

The presence of commonly occurring phenolic compounds gallic acid, gallic acid methyl ester, gallic acid ethyl ester, protocatechuic acid, 4-hydroxybenzoic acid, vanillin and 4-hydroxybezaldehyde were confirmed by HPLC using authentic samples

3.2 FLAVONOIDS-The flavones apigenin, luteolin, isothymusin, cirsimartin, orientin, isoorientin, isovitexin and crisilineol were isolated from the aerial parts of Os

3.3 PHENYL PROPANOIDS-Eugenol is one of the most widely distributed phenyl propanoid in the essential oil of Ocimum sactum leaves

3.4 NEOLIGNANS like Tulsinol A to Tulsinol G are found in the methanolic extracts of Tulsi leaves and are formed from the polymerisation of Eugenol [36].

3.5 COUMARIN-Ocimarin is one of the isolated Coumarin from leaves of Ocimum.

3.6 TERPENOIDS

Different terpenoids like sesquiterpenoids (β-caryophyllene), abietane diterpenoid (carnosic acid), oleane triterpenoids(oleanolic acid) and ursane triterpenoids

(ursolic acid)have been reported from Ocimum sanctum. The studies revealed ursolic acid as the most abundant terpenoid constituent in Ocimum sanctum. G ermacrene-A, trans- α -bergamotene are also found.

3.7 STEROIDS

Phytosterols β -sitosterol, β -sitosterol-3-O- β -D-glucopyranoside, stigmasterol and campesterol were isolated from leaves and stems of Os

3.8 ESSENTIAL OIL

Os essential oil is mainly composed of terpenoids including acyclic monoterpenoids, monocyclic terpenoids, bicyclic terpenoids, aliphatic aldehydes, phenolic acids, esters and sesquiterpenoids. The spicy-green-notes of Os essential oil is due to methyl eugenol, β -caryophyllene, β -caryophyllene oxide and germacrene D, while spicy-peppery-notes corresponds to germacrene D

3.9 FIXED OIL (non-volatile oil)

The fixed oil content in Os seeds was composed of mainly linoleic acid(Major), α -linolenic acid, oleic acid, palmitic acid and stearic acid.

3.10 FATTY ACID DERIVATIVE

Fatty acid derivatives like palmityl glucoside and sanctumoic acid were exhibited mosquitocidal activity, while cerebrosides showed antistress activity.

3.11 POLYSACCRIDE

A polysaccharide isolated from Os leaves contain monosaccharide compositions rhamnose (23.3%), xylose (19.2%), arabinose (42.2%), glucose (10.3%) and galactose (5.0%).

4 PHARMACOLOGICAL ACTIVITIES

Table 4.4 showing different pharmacological activities shown by different chemical constituents

S.No	Pharmacological	Chemical Constituent	Class of compound
	Activity		
1.	Anticancer	Orientin	Flavonoid
		α-bisabolol	Sesquiterpene alcohol
		Eugenol	Phenyl propanoid
		β -elemene	Sesquiterpene
		β-caryophyllene	
2.	Antioxidant	Rosmarinic acid	Phenolic acid
		Isothymusin	Flavonoid
		Isothymonin	Flavonoid
		Cirsimaritin	Flavonoid
		Cirsilineol	Flavonoid
		Eugenol	Phenyl propanoid
		β-Caryophyllene	Sesquiterpene
		α-Copaene	Sesquiterpene
		Chicoric acid	Phenyl propanoid
		δ-Cadinene	Bicyclic sesquiterpene
			Sesquiterpene
		Farnesene	Monoterpene
		Menthyl acetate	
3.	Antidiabetic	16-Hydroxy-4,4,10,13-	Triterpenoid
		tetramethyl-17-(4-	
		methyl-pentyl)-	
		hexadecahydro-	
		cyclopenta[a]-	
		phenanthren-3one	
4.	Antimicrobial	Orientin	Flavonoid
		δ-Cadinene	Bicyclic sesquiterpene

			Flavonoid
		Vicenin	
5.	Immunomodulation	Chicoric acid	Phenylpropanoid
6.	Anti inflammatory	Rosamarinic acid	Phenyl acid
		Isothymusin	Flavonoid
		Isothymonin	Flavonoid
		Cirsimaritin	Flavonoid
		Cirsilineol	Flavonoid
		Eugenol	Phenyl propanoid
		4',5-Dihydroxy-3',7,8-	Flavonoid
		trimethoxy flavone	
		Limonene	
		Bornyl acetate	Monoterpene
		Germacrene	Acetyl ester
		δ-Gurjunene	Sesquiterpene
			Cyclo aromoadendrane
			sesquiterpenoid
7.	Anti-Stress (Abiotic	α-bergamotene	Sesquiterpene
	stress)	Ocimarin	Coumarin
8.	Antifertility	Ursolic acid	Pentacyclic triterpenoid
9.	Memory enhancing	Methyl Eugenol	Phenylpropene
	activity		
10.	Radioactive property	Orientin	Flavonoid
		Vicenin	Flavonoid
11.	Antilipidemic activity	Eugenol	Phenylpropanoid
		Methyl Eugenol	Phenylpropene
12.	Anticoagulent activity	Guaiene	Sesquiterpene

4.1 Anticancer activity of Tulsi

Cancer has been the utmost cause of death in developing countries.[2] Tulsi is very effective in the treatment of cancer of various organ such as oral cavity, stomach, colon, liver, breast, oesophagus, intestine, pancreas etc. Anticancer property of Ocimum sanctum cannot be explained based on pharmacological activity of a single component. Synergistic effects of various phytochemical constituents (flavonoids, saponins, tannins and terpenoids) account for the anti-cancer property of the Tulsi.[37]The alcoholic extract of leaves of Tulsi has a modulatory influence on carcinogen metabolizing enzymes such as cytochrome P 450, cytochrome b5, glutathione S-transferase and these are important in detoxification of carcinogens.[8] The antioxidant and immunostimulant activity of Ocimum sanctum also work against the cancer growth in an indirect manner along with a direct cytotoxic effect towards the cancerous and other apoptotic cells. Ethanolic extract of Ocimum sanctum reduced the size of tumours cell and increase the lifetime of mice having sarcoma-180 solid tumours.[3]

The genesis of cancer is caused by numerous factors like excessive use of alcohol, exposure to environmental toxins, excessive exposure to sunlight, viruses, genetic problems etc. [38]. The paste form of Tulsi extracts shows satisfactory results in the prevention of 7,12-dimethylbenz(a) anthracene (DBMA) which causes buccal cavity. [39] N-methyl-A-nitro-N-nitroguanidine (MNNG), which is a nitroso compound is a gastric carcinogen. MNNG induces erosions of the gastric mucosa because it is a potent mutagen. MNNG intragastric administration increased cell proliferation and angiogenesis which then leads to cell carcinomas.[40] Alcoholic extract of Tulsi before controlling 7,12 dimethybenz[a] anthracene decreased phase I enzymes which result in the lower levels of lipid and protein oxidation and also increased in the levels of antioxidant and phase II enzymes.[41] Ursolic acid shows inhibiting activity towards tumorigenesis, tumor high promotion, and suppress angiogenesis.[42].It is found to non-toxic to normal cells.[43] By high performance thin layer chromatography it has been found that green variety of Tulsi has higher amount of ursolic acid than the black variety of Tulsi.[44]

4.2 Antioxidant activity

Tulsi is used to prevent the oxidation of lipids, sugars, proteins and DNA that may generate aldehydes, ketones, esters, and other products which can be toxic for the living organisms. Ocimum extract protects formation of radiation induced lipid peroxidation that protects the reduction of GSH (Glutathione) and other antioxidant enzymes.[45]

Tulsi acts as a powerful antioxidant due to present of Polyphenol Rosmarinic acid present in it. This acid prevents the excess oxidation because excess of oxidation in the body causes the cell damage. [46] The aqueous, hydroalcoholic and methanolic extracts of Ocimum sanctum show antioxidant property, both in vivo and in vitro. Oral feeding of it provides liver and aortic tissue protection from hypercholesterolemia induced peroxidative damage.[47] The Phenolic compounds e.g., cirsilineol, cisimaritin, isothymusin, apigenin and appreciable quantities of eugenol from Ocimum sanctum extract of fresh leaves and stems have very good antioxidant activity.[8]

Oleanic acid and ursolic acid which are present in Tulsi possess strong antioxidant activity and were shown to protect against Adriamycin induced lipid peroxidation in liver microsomes and shown to protect against damage caused by the ascorbic acid. [48] The extract of Tulsi with WR-2721 has increased protection against chromosome damage and the delayed chromosome toxicity of WR-2721 were also eliminated.[49] Tulsi enhances the basal activities of endogenous antioxidant enzymes and antioxidant capacity of HepG2 cells due to which there is decrease in ROS (reacting oxygen species). The extract of Tulsi was also found a potent scavenger of hydroxyl and superoxide radicals in vitro.[50]

4.3 Antidiabetic activity

Diabetes is a chronic disease which is also characterized by hyperglycaemia, hyperlipidaemia, hyper aminoacidemia. It leads to decrease in insulin secretion.[51]

Ocimum sanctum shows antidiabetic activity. Both floral and leafy parts can be used as antidiabetic activity because these inhibit carbohydrate hydrolysing enzymes. Ocimum sanctum is used in the treatment of diabetes related metabolic disorders and can be used in both in hypoglycaemic and hyperglycaemic activity.[1] Leaf extracts of Ocimum sanctum have stimulatory effects on the physiological pathways of insulin secretion from perfused pancreas, isolated islets, and clonal pancreatic cells.[52]

Its therapeutic potential as antidiabetic agent is due to the presence of eugenol which reduces elevated serum sugar, cholesterol triglyceride, lactate dehydrogenase, alanine transaminase, aspartate transaminase and alkaline phosphatase.[53]

4.4 Antimicrobial activity

The essential oil extracted from tulsi contains biologically active constituents that are insecticidal, nematocidal and fungistatic. It is used against many pathogens, and it is used in wound healing, treatment of skin infections and for the preservation of food stuff.[54] The phytoconstituents isolated from various parts of Ocimum sanctum inhibit the growth of the Klebsiella, E. coli, Proteus, Staphylococcus aureus, Vibrio cholerae.13] The tulsi extract

can act as an anti-microbial agent for the treatment of water. This treatment of water is ecofriendly, cost effective and easily accessible. As compared to chemical treatment ,Ocimum sanctum leaves has no side effect on human body [55].

4.5 Immunomodulation

Tulsi has very good immuno-enhancing properties that prepare the body from the attack of foreign elements like bacteria, viruses, microbes, allergens etc. and maintain the overall balance of the body.[56]

Tulsi enhances both cellular and humoral immunity. Ocimum sanctum L. aqueous extract reduces the bacterial total count and increases the count of neutrophil and lymphocyte and demonstrated a good phagocytic ability.[57] Tulsi shows immunomodulatory properties due presence of flavonoids in the extracts of Tulsi leaves.[58] Fungal endophytes which is the extract from roots of Ocimum sanctum can be used for in vitro immunomodulatory and free radical scavenging activity.[59]

4.6 Anti-inflammatory

Proteins have specific function within the living organisms which are guided by the complex structure. Due to several external and internal stressors, proteins lose their well-organized structure and they become non-functional. Protein denaturation causes inflammation. Ocimum sanctum has the ability to inhibit protein denaturation.[60]

Ocimum sanctum shows anti-inflammatory activity because it inhibits arachidonate metabolism supplemented by antihistaminic activity. [61] It shows anti-inflammatory action against carrageenan and many sanctum extract contains compounds like Cirsilineol, Cirsimaratin, Isothymonin, Apigenin, Rosamarinic acid and Eugenol which are responsible for its anti-inflammatory activity.[2]

4.7 Anti-stress activity

It can reduce toxicant stress, abiotic stress, metabolic stress and mental stress. It reduces the toxicant stress because it has the ability to prevent body organ like liver, kidney, brain etc against the cellular, genetic and immune damage caused by various chemicals.

The beneficial metabolic effects include the protection of the liver, kidneys', pancreatic cells ,enhancement of the bile acid formation and reduction in the liver lipid synthesis.[62]

4.8 Hepatopancreatic activity-

One of the most common and dangerous fish diseases in fish cultures is caused by parasitic protozoans. Hexamita sp is one of the most common protozoan diseases which causes medium to Severe deaths in the hatcheries. The infection increases the oxidative stress in the liver of the fish infected by the protozoa. When given different dosages of the dried powdered leaf to the infected fish, several antioxidant enzymes like Catalase, Glutathione peroxidase, Superoxide dismutase level increases and stress markers like Malondialdehyde levels decreases. The skin reformation is also accelerated in the scar region.[63]

The amount of vitamin C and vitamin E increases in the hepatopancreas as well as the sodium potassium levels increases when shrimp is given an herbal supplement.

4.9 Antifertility Activity

Ocimum sanctum has a potential ability to act as a contraceptive agent. In albino rats, Benzene extract of OS decreased the level of total sperm count and sperm motility. This was due to the anti-androgenic property of the OS leaves. This effect is reversible as sperm count becomes normal after the withdrawal of the treatment.[49] When similar treatment was given to the rabbit, decrease in the total sperm count was observed with increased testosterone levels and decreased LH- FSH levels.[64].

The long-term feeding (3months) of the OS leaves to the male albino rats decreased the weight of the reproductive organs like testis along with the decrease in total sperm count and sperm mobility.[65]

Aqueous extract of O. gratissimum causes destructive effects on the testis without causing any disruption of the testicular endocrine activity. It destroys the seminiferous epithelium followed by edema and spermatozoa arrest at different stages.[66]. The local women as well as ayurvedic physicians use tulsi leaves for antifertility effect which has negligible side effects. It reduces spermatogenesis in males by reducing the activity of Sertoli cells. In females it prevents the implantation of ovum. The major constituent responsible for antifertility is ursolic acid.[67]

4.10 Memory enhancing activity

Tulsi creates a fault against the Acetylcholine neurotransmitter which subsequently decreases the activity of the enzyme Acetylcholinesterase. Acetylcholine is responsible for the memory, maintaining sleep and promoting Rapid eye movement sleep (REM) therefore tulsi has the potential of increasing the level of acetylcholine transmission and hence developing the memory and functioning of the brain.[67].

4.11 Radio-protective properties: -

About 50% of cancer receiving radiation therapy during their treatment and it contributed about 40% of curative treatment for cancer.[68] However this radiation therapy also caused side effects due to radiation-induced damage to normal tissue.[69] By series of experimental studies it has been shown that Tulsi possess selective radioprotective effect at non-toxic concentrations.[70] The adverse effect of radioiodine exposure is due to generation of reactive

oxygen species which then damage the DNA.[71] The mechanism of action of Tulsi by free radical scavenging. However, scavenging of free radical is not possible pathway for radioprotection of Tulsi. Talalay et al. have suggested that phenolic antioxidants bind to the receptors which result in the increase in activity of detoxifying enzymes.[72] Studies have shown that genistein, reduced that radiation induced fibrosis without protecting the small tumors growing in the lung. [73] Similar type of mechanism may occur in Tulsi flavonoids.

4.12 Eye diseases

Tulsi leaf juice along with triphlala is used in eye drop preparations which is recommended for chronic conjunctivitis, glaucoma, and other eye diseases. About three drops of Tulsi oil along with honey is also supposed to improve the eyesight.[74] Muktadi Mahanjana prepared from Tulsi and various herbs is used in the treatment of pittaj eye disorders such as cataract, pterygium corneal ulcer etc. [75]

4.13 Antilipidemic activity

Atherosclerosis, Hyperlipidemia, and associated disorders are the big health concern now a days. It has been found that Tulsi has lower the levels for triglycerides, total cholesterol in acute triton WR1339-induced hyperlipidemia in rats.[76]

4.14 Anticoagulant Activity:

It has been studied that Tulsi (3ml/kg, ip) possesses anticoagulant activity. The blood clotting time was prolonged, and the response was comparable to aspirin (100 mg/kg). The reason is that Tulsi has anti-aggregatory action on platelets.[77]

5 PHYTOCHEMICAL EVALUATION

Qualitative phytochemical analysis of Tulsi: -

The samples of different species of tulsi were collected and then the leaves of these samples were cleaned and dried, powdered and stored in a bottle.

Preparation of Ocimum sanctum leaves extract: - Fresh leaves of Tulsi were softly eroded in deionized water to remove dust particles and dried under sunlight for seven days. Dried leaves are then grounded in mortar using a pestle. Then the powder was sieved to get fine particles of uniform size.

Preparation of Aqueous solvent extract: - 50 gm of the leaves after taking their powder form were soaked in 250 ml of distilled water, allowed to stand overnight and then filtered.

Preparation of Alcoholic solvent extract: - 30 gm of the powdered form of leaves were soaked in 200ml of alcoholic solvent (methanol) and allowed to stand overnight and then filtered.

5.1 Test for alkaloids: -Few drops of Mayor's reagent and concentrated hydrochloric acid were added to 2 ml of methanol extract. Presence of white precipitate or green colour indicate the presence of alkaloids. [78]

5.2 Test for flavonoids: - 10% solution of hydroxide was added to the sample then yellow colour appears and after addition of 1 ml of HCL the solution should become colourless.[79]

5.3 Test for saponins: - 1ml Crude extract was mixed with 5 ml water and it was shaken vigorously. The presence of saponins indicated by the formation of stable foam.[80]

5.4 Test for terpenoids: - 2 ml of chloroform was mixed with rude extract and dried it. Then 2 ml of concentrated hydrochloric acid was added to it and heated it for about 2 minutes. Greyish colour at the interface indicated the presence of terpenoids.[81]

5.5 Test for Phlobatannins: - Powder sample was mixed with distilled water then shaken well and filtered. Then 1% aqueous HCl was added to it and boiled with the help of hot plate stirrer.[82]

5.6 Test for glycoside: - To extract solution in glacial acetic acid, few drops of ferric chloride and sulphuric acid were added. Bluish green colour in the upper layer and a reddish-brown coloration at the junction of two layers were observed.[83]

5.7 Test for carbohydrate: - Take 2 ml extract in a test tube, then 1ml Fehling solution A and B are added to it and boiled for some time. Red brick precipitate is produced.[84]

Phytochemicals	Presence or absence in methanolic leaf extract	Presence or absence in aqueous leaf extract
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	-
Terpenoids	+	_
Phlobatannins	-	-
Glycoside	+	-
Carbohydrate	+	-

Table 5.5 Qualitative phytochemical analysis of methanolic and aqueous extract of tulsi

6 CONCLUSION AND FUTURE PROSPECTIVE

Tulsi has been used from very ancient time in Ayurveda because of its pharmacological importance. It can be concluded from above information that it is a very important drug. It can be used as an anticancer, antioxidant, antimicrobial, antidiabetic, and anti-inflammatory. It also possesses anti-stress activity, antifertility activity, anti-arthritis, radio protective effect, anthelminthic activity, antiaging effect etc. There are many herbal plants, but it has maintained the title of Queen of herbs due to its high potential. The main advantages of it in the various treatments includes the safety, lesser cost ,efficacy and availability throughout the world. There is a further requirement to research on the chemical aspects of Ocimum sactum to get newer molecules with new pharmacological potential and applications. The long and wide history of traditional uses, huge spectrum of pharmacological properties and the studies suggested

Ocimum sactum as a safe valuable herb for the clinical trials and applications. The compilation of chemical constituents along with their pharmacological properties will be quite helpful in future studies on Ocimum sactum plant as well as in discovery of new leads for the drugs. In the future, research may make a transgenic sanctum plant to eradicate the environmental and abiotic stress of the plant and also to address a particular chemical constituent acting against various human diseases. *O. sanctum* is the important gift of nature so "we should use it wisely and protect it".

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