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Research Article

Pharmacognostic and scientific evaluation of the plant- Tulsi (Ocimum sanctum)

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ABSTRACTS

The plant Tulsi is described vividly in Ayurved .Two types of Tulsi are described and their different Pharmacological actions and therapeutic uses are also described in detail. Pharmacognostic study of a plant not only helps to identify the plant among different species but also helps to differentiate the different types of the same plant. In the present study, review of the plant Tulsi was done from Ayurvedic texts and macroscopic and microscopic sections were taken to identify the species.

Keywords: Tulsi, pharmacognostic study

INTRODUCTION

Tulsi (Ocimum sanctum) is described in Ayurveda having many medicinal properties and a wide therapeutic range. It is used specially in the management of kasa (cough), Shwasa (Ashtma), Jwara (fever) and pratishaya (common cold). In India, the herb Tulsi also called holy basil (sometimes spelled "Tulasi") has been widely known for its health promoting and medicinal value for thousands of years. Commonly called sacred or holy basil, it is a principal herb of Ayurveda, the ancient traditional holistic health system of India. Holy basil is also known as "The Incomparable One", "The Mother Medicine of Nature", and "The Queen of Herbs".

Tulsi is identified by botanists primarily as Ocimum sanctum (Rama and Krishna Tulsi varieties) or more recently Ocimum tenuiflorum, and Ocimum gratissimum (Vana Tulsi variety). Belonging to the Lamiaceae/Labiatae mint family, these and other closely related species and varieties (e.g., Ocimum canum) are cousins of the familiar sweet basil cooking herb Ocimum basilicum. In parts of India, all of the basils are honoured as Tulsi[1].

The brief introduction of the plant Tulsi is as below:

Scientific Classification[2]

Kingdom: Plantae

(unranked) Angiosperms

(unranked) Eudicots
(unranked) Asterids
Order: Lamiales
Family: Lamiaceae
Genus: *Ocimum*
Species: *O. tenuiflorum*
Binomial name: *Ocimum tenuiflorum* or *Ocimum sanctum* L.

Part used	:	Panchang
Vernacular names	:	
Hindi	:	Tulsi, Tulas, Vrindha, Virandra
Gujarati	:	Tulsi, Tulsi
English	:	Holy Basil, Sacred Basil, Mon'k's Basil, whitebasel, Mosquito plant of south Africa.
Marathi	:	Tulasa, Surasa, Tulas

Vargikarana or classification [3-4]

Swasahara Mahakashaya	:	According to Charaka
Surasadi, Shirovirechana	:	According to Madanpal Nighantu
Puspa varga	:	According to Bhavaprakash

Rasa panchaka [5-6]**:**

Rasa = Katu, Tikta & Kashaya

Guna = Laghu, Ruksha, Tikshna

Veerya = Ushna

Vipaka = Katu

Prabhava = Krimighna

Doshaghnata and Karma: [7]**Kapha** vata Shamaka (Pacifies Kapha and vata doshas) & Jantughna (antiseptic)

Property & guna karma[8] **:** Vedanahara,Pachana ,Twagdoshahara, Anulomana , Sirovirecana , Krimighana , Aksapanasanraka ,Hridya , Raktashodhaka ,Kasahara ,Ksayanasaka , Swasahara

PHARMACOGNOSTIC STUDY[9-10]:

Habit and habitat[11-12]**:** An erect herbaceous, much branched, softly hairy annual 30-75 cm, high found throughout India, ascending upto 1800 mt in Himalayas and Andaman Nicobar islands. It is considered sacred by Hindus. The plant is commonly cultivated in garden and also grown near temples. It is propagated by seeds. Tulsi, now - days is cultivated commercially for its volatile oil.

Material- Acharya Charaka has considered Tulsi under Shwashara Mahakashaya[13]. Hence, for the present study Tulsi leaf, steam and root was used.

Collection of samples: Tulsi leaf, steam and root were collected from Jamnagar.

Processing and Preservation: The collected Panchang was preserved in a solution of Formalin - Aceto Alcohol (FAA) Macroscopic and Microscopic Characters of the plant was studied systematically by taking transverse section. The plant was subjected for microscopic examination. Photomicrographs of all the section were also prepared. Diagnostic features were studied and presented here.

Botanical description of the plant (**Figure 1 and 2**)

Figure 1

Figure 2

Root: Thin, wiry, branched, hairy, soft, greenish brown externally & Pale blackish internally

Stem: Erect, herbaceous, woody, branched, hairy, subquabrangular, externally greenish, internally cream coloured, fractured, fibrous, in barks & short in xylem, odour - faintly aromatic.

Leaf: Leaves (Green type of O. sanctum), exstipulate, opposite, petiolate. Petiole 2.5 to 3.0 cm length, slender, thin, pubescent with narrow adaxial groove, lamina elliptical to ovoid, oblong 5-6 cm length, and 2.5 cm to 3 cm breadth, pubescent, margin entire, irregularly undulated or bluntly serrate, apex acute or obtuse, adaxial surface bright green, with prominent veins, venation pinnately reticulate with 5-6 alternate paits of lateral veins, glandular dots seen minutely on the abaxial side; odour - aromatic, & taste - pungent.

Flower : Crimson coloured, small inclose whorls, bracts about 3 mm long and broad, pedicles longer than calyx & slender, pubescent calyx ovoid or campanulate, 3-4 mm bilipped, upper lip broadly obovate or sub orbicular, shortly apiculate, lower lip longer than upper having form nucronate teeth, lateral two short and central two largest, corolla 4 mm long, pubescent, odour, aromatic, taste pungent.

Fruit : A group of 4 nut lets, each with one seed, enclosed in and enlarged, membranous, veined calyx, nut lets subglobose or broadly elliptic, slightly compressed, near smooth, pale brown or reddish with small black marking at the place of attachment to the thalamus, odour-aromatic, taste pungent.

Seed:Rounded to oval brown mucilaginous when soaked in water; 0.1 cm long, slightly notched at the base, no odour, taste-pungent, slightly mucilaginous.

Microscopic characters: (Fig. No: 4, 5, 6)

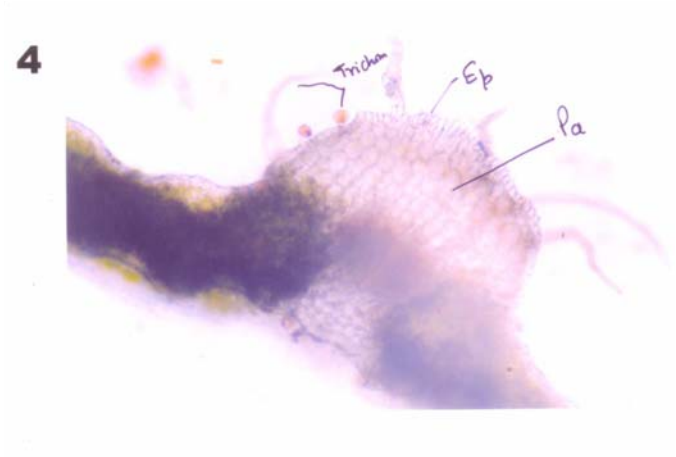

Fig.No.4

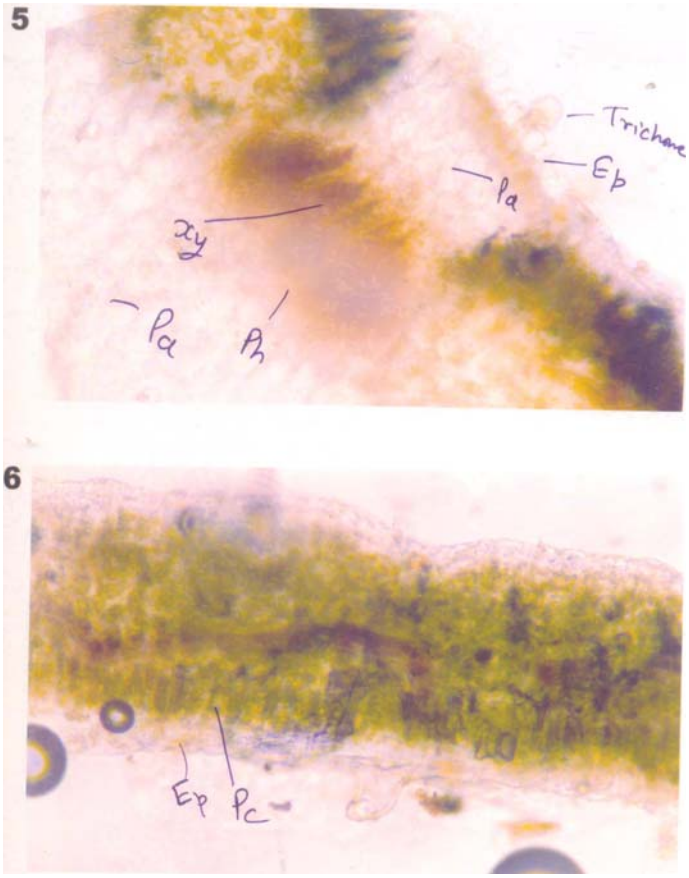

Fig.No:5, 6

TRANSVERSE SECTION

Leaf: ***Midrib*** – Shows somewhat cordate outline, consisting of single layered epidermis composed of thin walled, oval cells having a number of covering and glandular trachomes covering trichomaes multicellular 1-7 celled long, rarely slightly reflexes at tip, glandular trichomas short, sessile with small stalk and 2-8 celled ballon shaped head, measuring 22-27 in diameters, epidermis followed by 1 or 2 layers and 3 to 5 layers of thin walled, elongated, parenchyma cells towards upper and lower surfaces respectively, vascular bundles situated centrally.

Lamina: Leaf contains single layers of pallaside cells which indicate that leaves are of dorsiventral nature. Epidermis, trichomes are similary to those of midrib, both anomocytic and diacytic types of stomata present on both surface, slightly raised above the level of epidermis, pallaside single layered followed by 4-6 layers of closely packed spongy parenchyma with chloroplast

Stem: Shows somewhat cylindrical in shape. Upper surface having covering multicellular glandular trichomas, where covering multicellular trichomas have 5-12 cells. Single layered epidermis, 5 to 9 layers of Parenchymateous cells in cortex region with fibre patches also available. Fibre made by sclerenchyma with its stain by phlorogucinal and HCl.

Vascular bundles are available an periphery. Outerside phloem and inner side xylem, where xylem having vessels, tracheids which are stain by phlorogucinol and HCl. Centre portion is pith it's contain parenchymatous cells.

Root: One to five layers of small cells, some cells are stratified and unlignified. Nine to twelve rows of tangentially elongated to isodiameteric parenchyma cells of cortex region contain some small groups to bigger groups of fibres. Vascular bundles contain phloem, vessels, tracheids and medullary rays. Medullary rays run radially from the center to the cortex through the phloem one to two cells in width.

CHEMICAL CONSTITUENTS[14-19]

Tulsi leaves contain bright, yellow coloured & pleasant odour of volatile oil (0.1 to 0.9%). The oil content of the drug varies depending upon the type, the place of cultivation & season of its collection. The oil is collected by steam distillation method from the leaves. It contains - Eugenol (70%) Carvacrol (3%), Eugenol methyl ether (20%) Caryophyllin, linalool, Aneole, Chavicol, nerol, terpinin 4 - 01, decylaldehyde, r-selinene, α & β - pinenes, champhor sesequiterpenes.

The plant is used as a pot herb; leaves are used as condiment in salads and other foods. It is also reputed to have medicinal properties. Besides the volatile oil, the plant is reported to contain alkaloids, glycosides, saponins and tannins. The leaves contain ascorbic acid carotene, appreciable amount of Vitamin C, traces of maleic acid, citric and tartaric acid

PHARMACOLOGICAL ACTION[20-21]

Leaf extract enhanced anti SRBC haemagglutination titre and IgE antibody titre, as measured by passive cutaneous anaphylaxis in rats; antigen- induced histamine release from peritoneal mast cells of sentisized rats in vitro was significantly inhibited by it, it also antigonised responses to various spasneogins by isolated guinea pig ileum. Thus, leaf extract modulates humoral immune response.Extract of leaves also showed highly significant clinical and biochemical clearance of viral hepatitis in 14 days of treatment.

The oil is reported to posses antibacterial & iinsecticidal properties. It inhibits the invitro growth of Mycobacterium tuberculosis and Micrococcus pyogens var. curves:in antitubercular activity, it has one-tenth the potency of streptomycin and one –fourth that of isoniazid. It has marked insecticidal activity against mosquitoes, though it is not comparable to that of pyrethrum, the mosquito repellent action lasts for two hours. The oil is also active against Salmonella typhosa.

The juice of leaves possess diaphoretics, antispasmodic, antiperiodic. Stimulating and expectorant properties: it is used to catarrh and bronchitis, applied to the skin in ringworm and other cutaneous disease and also in earache. An infusion of the leaves is used as a stomachic in gastric disorders of Children.

PRECLINICAL STUDIES

Antidiabetic: Ethanolic extract of *O. sanctum* L. significantly decreases the blood glucose, glycosylated hemoglobin and urea with a concomitant increase in glycogen, hemoglobin and protein in streptozotocin-induced

diabetic rats[22]. This extracts also resulted in an increase in insulin and peptide levels and glucose tolerance. The constituents of *O. sanctum* L. leaf extracts have stimulatory effects[23] on physiological pathways of insulin secretion, which may underlie its reported antidiabetic action. Grovel *et al.* suggested that treatment with *O. sanctum* L. extract for 30 days to normal rats fed with fructose for 30 days significantly lowered serum glucose level[24] in comparison with control group. However, *O. sanctum* L. extract has no significant effect on hyperinsulinemia. Ghosap *et al.* unravel the possible mechanism[25] of glucose-lowering activity of *O. sanctum* L. in male mice.

The study suggested that *O. sanctum* L. decreases the serum concentration of both cortisol and glucose and also exhibited antiperoxidative effect. Therefore *O. sanctum* L. may potentially regulate corticosteroid- induced diabetic mellitus. In another study the effect of *O. sanctum* L. on three important enzymes of carbohydrate metabolism [glucokinase (gk), hexokinase (hk) and phosphofructokinase (PFK) along with glycogen content of insulin-dependent (skeletal muscle and liver) and insulin-independent tissues (kidneys and brain) was studied by Vats *et al*[26], in streptozotocin (STZ, 65 mg/kg)-induced model of diabetes for 30 days in rats. Administration of *O. sanctum* L. *extracts* 200 mg/kg for 30 days lead to decrease in plasma glucose levels by approximately 9.06 and 24.4% on 15th and 30th day. *O. sanctum* L. significantly decreased renal but not liver weight (expressed as % of body weight) *O. sanctum* L. glycogen content in any tissue; also *O. sanctum* L. partially corrected the activity of glucokinase (gk), hexokinase (hk) and phosphofructokinase (PFK) distributed in the diabetic control.

Tlsi (*O. sanctum* L.) leaf powder[27] was fed at the 1% level in normal and diabetic rats for a period of one month and the result indicated a significant reduction in fasting blood sugar urogenic acid, total amino acids level. This observation indicates the hypoglycemic effect of *O. sanctum* L. in diabetic rats. Chattopadyay also reported that oral administration of alcoholic extract of leaves of *O. sanctum* L. led to marked lowering of blood sugar [28] level in normal, glucose-fed hyperglycemic and streptozotocin-induced diabetic rats. Furthermore, the extract potentiates the action of exogenous insulin in normal rats. The activity of the extract was 91.55 and 70.43% of that of Tolbutamide in normal and diabetic rats, respectively.

Cardiac activity: Oral feeding of hydroalcoholic extract of *O. sanctum* L. (100 mg/kg) to male Wister rats subjected to chronic-resistant stress (6 h/day for 21 days) significantly prevented the chronic-resistant stress/induced rise in plasma cAMP level, myocardial superoxide dismutase and catalase activities [29] as well as the light microscopic changes in the myocardium. Wister rats fed with fresh leaf homogenate of *O. sanctum* L. (50 and 100 mg/kg body weight) daily 30 days inhibit isoproterenol-induced changes[30]in myocardial superoxide dismutase, glutathione peroxidase and reduced glutathione.

In another study effect of pre- and co-treatment of hydroalcoholic extract of *O. sanctum* L. at different doses (25, 50, 75, 100, 200 and 400 mg/kg) was investigated against isoproterenol (ISO, 20 mg/kg, Sc) myocardial infarction [31] in rats. *O. sanctum* L. at the dose of 25, 50, 75 and 100 mg/kg significantly reduced glutathione (GSH), superoxide dismutase and LDH levels. In this study, it was observed that *O. sanctum* L. at the dose of 50 mg/kg was found to demonstrate maximum cardioprotective effect. The generation of drug-induced oxygen radicals in heart cells led to cardiac lipid [32] membrane peroxidation. Urosolic acid (UA) isolated from *O. sanctum* L. have been identified as a protector against Adriamycin (ADR)-induced lipid peroxidation. Protection with UA was 13 and 17% in liver and heart microsomes, respectively. On combination with oleanolic acid (OA) isolated from *Eugenia jumbolata* , it increased to 69%.

Wound healing activity: Shetty *et al,* [33] evaluated the wound healing effect of aqueous extract of *O. sanctum* L. in rats. Wound-breaking strength in incision wound model, epithelization period and percent wound concentration in excision wound model were studied owing to increased per cent wound contraction. *Ocimum sanctum* L. may be useful in the management of abnormal healing such as keloids and hypertropic scars. Ethanolic extract of leaves of *O. sanctum* L. was investigated for normal wound healing and dexamethasone-depressed healing[34]. The

extract significantly increased the wound breaking strength, wound epithelializes fast and wound contraction was significantly increased along with increase in wet and dry granulation tissue weight and granulation tissue breaking strength. The extract also significantly decreases the anti-healing activities of dexamethasone in all wound healing models.

Radio-protective effect: Radio-protective effect[35] of aqueous extract of O. sanctum L. (40 mg/kg, for 15 days) in mice exposed to high doses (3.7 MBq) of oral 131 iodine was investigated by studying the organ weights, lipid peroxidation and antioxidant defense enzyme in various target organs like liver, kidney, salivary glands and stomach at 24 h after exposure. Pretreatment with O. sanctum L. in radioiodine-exposed group showed significant reduction in lipid peroxidation in both kidney and salivary glands. In liver, reduced glutathione (GSH) levels showed significant reduction after radiation exposure while pretreatment with O. sanctum L. exhibited less depletion in GSH level even after 131 iodine exposure. However, no such changes were observed in the stomach. The results indicate the possibility of using aqueous extract of O. sanctum L. for ameliorating 131 iodine induced damage to the salivary gland. Two polysaccharides isolated from O. sanctum L. could prevent oxidative damage [36] to liposomal lipids and plasmid DNA induced by various oxidants such as iron, AAPH and gamma radiation.

Vrinda et al. reported that two water-soluble flavonoids, Orientin (Ot) and Vicenin (Vc), isolated from the leaves of O. sanctum L. provide significant protection against radiation,[37]lethality and chromosomal aberration in vivo. In order to select the most effective drug concentration, fresh whole blood was exposed to 4 Gy of cobalt-60 gamma radiation with O. sanctum L. without a 30 min pretreatment with 6.25, 12.5, 15, 17.5 and 20 micron of Ot/Vc in micronucleus test. Radiation significantly increased the micronucleus (MN) frequently. Pretreatment with either Ot or Vc at all concentration-dependent manner, with optimum effect at 17.5 µm.

The effect of aqueous extract (OE) of leaves of *O. sanctum* L. against radiation lethality [38] and chromosome damage was studied by radiation-induced lipid peroxidation in liver. Adult Swiss mice were injected with 10 mg/kg of gamma radiation 30 min after last injection. Glutathione (GSH) and the antioxidant enzymes glutathione transferase (GST), reductase (GSRx), peroxidase (GSPx) and superoxide dismutase (SOD) as well as lipid peroxide (LPx) activity were estimated in the liver at 15 min, 30 min, 1, 2, 4 and 8 h post-treatment. Aqueous extract itself increased the GSH and enzymes significantly above normal level, whereas radiation significantly reduced all the values and significantly increased the lipid peroxidation rate, reaching a maximum value at 2 h after exposure (3.5 times of control). Aqueous extract significantly reduced the lipid peroxidation and accelerated recovery to normal levels. In a comparative study[39] of radioprotection by ocimum flavonoids and synthetic aminothiol protectors in mouse showed Ocimum flavonoids as promising human radiation protectant. In this study, adult Swiss mice were injected intraperitoneally with 50 µg/kg body weight of Orientin (OT) or vicenin (Vc) 20 mg/kg body weight of 2-ercaptopropionyl glycine (MPG) 150 mg/kg body weight of WR2721 and exposed to whole body irradiation of 2 Gy gamma radiation 30 min later. After 24 hours, chromosomal aberrations were studied in the bone marrow of the femur by routine metaphase preparation after colchicines treatment. Pretreatment with all the protective compounds resulted in a significant reduction in the percentage of aberrant metaphases. Vicenin produced the maximum reduction in per cent aberrant cells while MPG was the least effective; OT and WR-2721 showed an almost similar effect.

Ganasoundari et al. [40] investigated the radio-protective effect of the leaf extract of *O. sanctum* L. (OE) in combination with WR-2721 (WR) on mouse bone marrow. Adult Swiss mice were injected intraperitoneally with OE (10 mg/kg for five consecutive days) alone or 100-400 mg/kg WR (Single dose) *O. sanctum* L. combination of the two and whole body was exposed to 4.5Gy gamma irradiation (RT). Metaphase plates were prepared from femur bone marrow on days 1, 2, 7 and 14 post-treatment and chromosomal aberrations were scored. Pretreatment with OE or WR individually resulted in a significant decrease in aberrant cells as well as different types of aberrations. The combination of the two further enhanced this effect; resulting in a two-fold increase in the protection factors (PF = 6.68) compared to 400 mg/kg WR alone.

Genotoxicity: *In vivo* cytogenetic assay [41] in Allium cepa root tip cells has been carried out to detect the modifying effect of *O. sanctum* L. aqueous leaf extract against chromium (Cr) and mercury (Hg)-induced genotoxicity. It was observed that the roots post-treated with the leaf extract showed highly significant recovery in mitotic index (MI) and chromosomal aberrations. When compared to pre-treated (Cr/Hg) samples, the lower doses of the leaf extract were found to be more effective than the higher doses. Immu-21, a poly-herbal formulation containing *O. sanctum* L. and other herbal extracts when given at 100 mg per kg daily over 7 days and 300 mg/kg daily over 14 days inhibited both cyclophosphamide (40 mg/kg i.p.)-induced classical and non-classical chromosomal aberration[42] (40-60% of control). This also reduces the increase in micronuclei in the bone marrow erythrocytes of mice treated with cyclophosphamide.

Antioxidant: The antioxidant capacity of [43] essential oils obtained by steam hydrodistillation from *O. sanctum* L. was evaluated using a high-performance liquid chromatography (HPLC) based hypoxanthine xanthine oxidase and OPPH assays. In hypoxanthine xanthine oxidase assay, strong antioxidant capacity was evident from *O. sanctum* L. (IC_{50} = 0.46 µL/ml).

In another study the aqueous extract of *O. sanctum* L. significantly increases the activity of anti-oxidant [44.] enzymes such as superoxide dismutase, catalase level in extract-treated group compared to control. Aqueous extract of *O. sanctum* L. inhibit the hypercholesterolemia-induced [45] erythrocyte lipid peroxidation activity in a dose-dependent manner in male albino rabbits. Oral feeding also provides significant leaver and aortic tissue protection from hypercholestrolemia-induced peroxidative damage. The effect of methanolic extract of *O. sanctum* L. leaves in cerebral reperfusion injury[46] as well as long-term hypoperfusion was studied by Yanpallewar *et al*. *Osimum sanctum* L. pretreatment (200 mg/kg/day for 7 days) prevented reperfusion-induced rise in lipid peroxidation and superoxide dismutase. *Osimum sanctum* L. pretreatment also stabilized the levels of tissue total sulfhydryl group during reperfusion.

Hypolipidemic : Administration of *O. sanctum* L. seed oil (0.8 gm/kg body weight/day) for four weeks, in cholesterol-fed (100 mg/kg body weight/day) rabbits significantly decreases serum cholesterol, triacylglycerol and LDL + VLDL cholesterol as compared to untreated cholesterol-fed group suggesting the hypo-cholesterolemic activity of *O. sanctum* L. Fresh leaves of *O. sanctum* L. mixed OS 1 and 2 g in 100 gm of diet given for four weeks brought about significant changes in the lipid [47] of normal albino rabbits. This resulted in significant lowering in serum total cholesterol, triglyceride, phospholipids and LDL-cholesterol level and significant increase in the HDL-cholesterol and total fecal sterol contents.

Antimicrobial: Singh *et a.l*[48] in his study suggested that higher content of linoleic acid in *O. sanctum* L. fixed oil could contribute towards its antibacterial activity. The oil show good antibacterial activity against *Staphylococcus aureus*, *Bacillus pumius* and *Pseudomonas aeruginosa*, where *S. aureus* was the most sensitive organism. Geeta *et al.* [49] studied that the aqueous extract of *O. sanctum* L. (60 mg/kg) show wide zones of inhibition compared to alcoholic extract against *Klebsiella*, *E. coli*, *Proteus*, *S. aureus* and *Candida albicans* when studied by agar diffusion method. Alcoholic extract showed wider zone for *Vibrio cholerae*.

Effect on gene transcription: The genes that have direct role in artherogenesis include LDRL, LxRalpha, PPARs, CD-36 because these genes control lipid metabolism, cytotoxin production and cellular activity within the arterial wall. To know whether or not the polyphenols extracted from *O. sanctum* L. have any effect on the transcription[50] of these genes, Kaul *et al.* cultured human mononuclear cells in the presence of polyphenols extracted from *O. sanctum* L. Transcriptional expression of these genes was measured by using RT-PCR and SCION IMAGE analysis software. These polyphenolic extracts were found to have the inherent capacity to inhibit the transcriptional expression of these genes.

Gastroprotective: The standardized methanolic extract of leaves of *O. sanctum* L. (OSE) given in doses of 50-200 mg/kg orally twice daily for five days showed dose-dependent ulcer protective effect against cold-restraint stress-induced gastric ulcers. Optimal effective dose (100 mg/kg) of OSE showed significant ulcer protection [51]

against ethanol and pyloric ligation induced gastric ulcer but was ineffective against aspirin-induced ulcer. OSE (100 mg/kg) also inhibits the offensive acid pepsin secretion and lipid peroxidation and increases the gastric defensive factors like mucin secretion, cellular mucus and lifespan of mucosal cells. Dharmani *et al.* evaluated the anti-ulcerogenic activity[52] in cold-restraint (CRU), aspirin (ASP), alcohol (Al), pyloric ligation (PL) induced gastric ulcer models in rats, histamine-induced (HST) duodenal ulcer in guinea pigs and ulcer healing activity in acetic acid induced (AC) chronic ulcer model. *Osimum sanctum* L. at a dose of 100 mg/kg was found to be effective in CRU (65.07%), ASP (63.49%), Al (53.87%), PL (62.06%) and HST (61.76%) induced ulcer models and significantly reduced free, total acidity and peptic activity by 72.58, 58.63 and 57.6%, respectively, and increased mucin secretion by 34.61% conclusively *Osimum sanctum* L. could act as a potent therapeutic agent against peptic ulcer disease. The antiulcerogenic [53] property of *O. sanctum* L. was studied in pyloric-ligated and aspirin-treated rats. The extract of reduced ulcer index, free and total acidity on acute and chronic administration seven days pretreatment increased the mucus secretion also. So it may be concluded that *O. sanctum* L. extract has anti-ulcerogenic property against experimental ulcers and it is due to its ability to reduce acid secretion and increase mucus secretion.

Immunomodulatory effect: Immunotherapeutic potential [54] of aqueous extract of *O. sanctum* L. leaf in bovine sub-clinical mastitis (SCM) was investigated after intramammary infusion of aqueous extract. The results revealed that the aqueous extract of *O. sanctum* L. treatment reduced the total bacterial count and increased neutrophil and lymphocyte counts with enhanced phagocytic activity and phagocytic index. In another study, the immunomodulatory effect of *O. sanctum* L. seed oil (OSSO) was evaluated in both non-stressed and stressed animals. [55] *Osimum* sanctum L. seed oil (3 ml/kg, Ip **)** produced a significant increase in anti-sheep red blood cells (SRBC) antibody titer and a decrease in percentage histamine release from peritoneal mast cell of sensitized rats (humoral immune responses) and decrease in food pad thickness and percentage leucocyte migration inhibition (cell-mediated immune responses). Co-administration of diazepam (1 mg/kg, Sc), a benzodiazepine (BZD) with OSSO (1 mg/kg, IP) enhanced the effect of OSSO on resistant stress induced changes in both humoral and cell-mediated immune responses. Further, flumazenil (5 mg/kg, IP) a central BZD receptor antagonist inhibited the immunomodulatory action of OSSO on resistant stress induced immune responsiveness. Thus, OSSO apparatus to modulate both humoral and cell-mediated immune responsiveness and these immunomodulatory effects may be mediated by GABAnergic pathway. Godhwani *et at.* [56] investigated the immunoregulatory profile of methanolic extract and an aqueous suspension of *O. sanctum* L. leaves to antigenic challenge of *Salmonella typhosa* and sheep erythrocytes by quantifying agglutinating antibodies employing the Widal agglutination and sheep erythrocyte agglutination tests and E-rosette formation in albino rats. The data of the study indicate an immunostimulation of humoral immunogenic response as represented by an increase in antibody titer in both the Widal and sheep erythrocyte agglutination tests as well as by cellular immunologic response represented by E-rosette formation and lymphocytosis.

Sexually transmitted disease: Extract of *O. sanctum* L. caused inhibition of *Neisseria gonorrhoeae* clinical isolates[57] and WHO organization strains. The activity is comparable to penicillin and ciprofloxacin.

Effect on central nervous system (CNS) : Different extracts of stem, leaf and stem callus (induced on slightly modified Murashige and Skoog's medium and supplemented with 2,4-dichlorophenonyacetic acid and kinetin) were tested for anticonvulsant activity [58] by maximal electroshock model using Phenytoin as standard. It was observed that ethanol and chloroform extractives of stem, leaf and stem calli were effective in preventing tonic convulsions induced by transcorneal electroshock. Ethanolic extract of leaves of *O. sanctum* L. prolonged the time of lost reflex in mice due to pentobarbital,[59] decreased the recovery time and severity of electroshock and pentylenetetrazole-induced convulsions and decreased apomorphine-induced fighting time and ambulation in 'open field' studies. In the forced swimming behavioral despair model, the extract lowered immobility in a manner comparable to Imipramine. This action was blocked by haloperidol and sulpiride, indicating a possible action

involving dopaminergic neurons. In similar studies, there was a synergistic action when the extract was combined with bromocriptine, a potent D2-receptor agonist. Nootropic agents are a new class of drugs used in situations where there is organic disorder in learning abilities.

Joshi and Parle [60] assessed the potential of *O. sanctum* L. extract as a nootropic and anti-amensic agent in mice. Aqueous extract of derived whole plant of *O. sanctum* L. ameliorated the amensic effect of scopolamine (0.04 mg/kg), diazepam (1 mg/kg) and aging-induced memory deficits in mice. Elevated plus maze and passive avoidance paradigm served as the exteroceptive behavioral models. *O. sanctum* L. extract decreased transfer latency and increased step-down latency, when compared to control (piracetam-treated), scopolamine and aged groups of mice significantly. So *O. sanctum* L. preparation could be beneficial in the treatment of cognitive disorders such as dementia and Alzheimer's disease.

Methanolic extract of *O. sanctum* L. root extract[61] at a dose of 400 mg/kg (ip increases the swimming time of mouse in a despair swim test model, suggesting a central nervous system stimulant and/or anti-stress activity of *O. sanctum* L.

Antinociceptive (Analgesic): The analgesic[62] activity of alcoholic leaf extract of *O. sanctum* L. (50, 100 mg/kg, ip; 50, 100, 200 mg/kg, po) was tested in mice using glacial acetic acid induced writhing test. *O. sanctum* L. reduced the number of writhes. *Osimum sanctum* L. (50, 100 mg/kg ip) also increased the tail withdrawal latency in mice.

Anti-fertility: Benzene extract of *O. sanctum* L. leaves have a reversible anti-fertility [63] effect, as *O. sanctum* L. extract (250 mg/kg body weight) for 48 days decreases the total sperm count, sperm motility and forward velocity. The percentage of abnormal sperm increased in caudal epididymal fluid and the fructose content decreased in the caudal plasma of the epididymis and the seminal vesicles. All these parameters returned to normal two week after the withdrawal of the treatment.

Anthelmintic activity: The anthelmintic activity[64] of the essential oil from *O. sanctum* L. was evaluated by Caenorhabditis elegance model. Eugenol exhibited an ED^{50} of 62.1 µg/ml and being the predominant component of the essential oil, it was suggested as the putative anthelmintic principle.

Anti inflammatory: Compounds isolated from *O. sanctum* L. extract, Civsilineol, Civsimavatine , Isothymonin, Apigenin, Rosavinic acid and Eugenol were observed for their anti-inflammatory activity[65] or cyclooxygenase inhibitory activity. Eugenol demonstrated 97% cyclooxygenase-1 inhibitory activity when assayed at 1000 µM concentration (pn). Civsilineol, Civsimavitin, Isothymonin, Apigenin and Rosavinic acid displayed 37, 50, 37, 65 and 58% cyclooxygenase-1 inhibitory activity, respectively, when assayed at 1000 µM concentrations. The activities of these compounds were comparable to Ibuprofen, Naproxen and aspirin at 10, 10 and 1000 µM concentrations. Singh in his study[66] reported that linoleic acid present in different amount in the fixed oil of different species of *O. sanctum* L. has the capacity to block both the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism and could be responsible for the anti-inflammatory activity. A methanolic extract [67] and an aqueous suspension of *O. sanctum* L. (500 mg/kg) inhibited acute as well as chronic inflammation in rats as tested by carrageenin-induced pedal edema and cratonoil -induced granuloma and exudates, respectively, and the response was comparable to the response observed with 300 mg/kg of sodium salicylate. Both the extract and suspension showed analgesic activity in mouse hot plate procedure, and the methanol extract caused an increase in tail withdrawal reaction time of a sub-analgesic dose of morphine. Both preparations reduced typhoid-paratyphoid A-B vaccine-induced pyrexia. The antipyretic action of methanol extract and aqueous suspension was weak and of shorter duration than that of 300 mg/kg sodium salicylate.

Anticancer: Fresh leaf paste (topically) aqueous and ethanolic extract (orally) for their chemopreventive activity against 7, 12-dimethylbenzaanthracene (DMBA) induced (0.5%) hamster buccal pouch[60] carcinogenesis. Incidence of papillomas and squamous cell carcinomas were significantly reduced and increased the survival rate

in the topically applied leaf paste and orally administered extracts to animals. Histopathological observation made on the mucosa confirmed the profound effect of the orally administered aqueous extract than other. Prasbar *et al.* in their study reported that *O. sanctum* L. leaf extract blocks or suppresses the events associated with chemical carcinogenesis [68] by inhibiting metabolic activation of the carcinogen. In this study, primary cultures of rat hepatocytes were treated with 0-500 µg of *O. sanctum* L. extract for 24 h and then with 7,12-dimethaylbenz[a] anthracene (DMBA, 10 or 50 µg) for 18 h. Cells were then harvested and their DNA was isolated and analyzed by 32p post-labeling. A significant reduction in the levels of DMBA/DNA adducts was observed in all cultures pretreated with *O. sanctum* L. extract. Hepatocytes that were treated with the highest dose of extract (500 µg) showed a maximum reduction of 93% in the mean values of DMBA/DNA adducts. This suggests the inhibition of metabolic activation of carcinogen.

The chemopreventive activity [69] of seed oil of *O. sanctum* L. was evaluated against subsequently injected 20-methyl cholanthrene-induced fibrosarcoma tumors in the thigh region of Swiss albino mice. Supplementation of maximal-tolerated dose (100 µl/kg body wt.) of the oil significantly reduced 20-methaylcholathrene-induced tumor incidence and tumor volume. The enhanced survival rate and delay in tumor incidence was observed in seed oil supplemented mice. Liver enzymatic, non-enzymatic antioxidants and lipid peroxidation end product, malondialdehyde level were significantly modulated with oil treatment as compared to untreated 20-methylcholathrene injected mice. The chemopreventive efficacy of 100 µl/kg seed oil was comparable to that of 80 mg/kg vita-E.

Thyroid activity: The extract of *O. sanctum* L. leaf extract (OSE) on the changes in the concentrations of serum Triiodothyronine (T3), Thyronine (T4) and serum cholesterol were investigated. [70] OSE at the dose of 0.5 g/kg body weight for 15 days significantly decreased serum T4 concentration; however, no marked changes were observed in serum T3 level, T3/T4 ratio and in the concentration of serum cholesterol. It appears that OSE is antithyroidic in nature.

THERAPEUTIC USES [71-73]

Swasa, Kasa, Hikka, Chardi, Krimi, Parsvasula, Kushta, Asmari, Metraroga, Aruchi, Pratisyaya. The plant is reported to possess anabolic, hypoglycemic, smooth muscle relaxant, cardiac depressant, anti fertility ,adapotogenic & immunomodulatory properties.

(i). Antimicrobial effect [74,75]: Essential oil of Tulsi has antibacterial, antifungal & antiviral properties. If inhibits the growth of E Coli, B. anthrac, M. tuberculosis etc.Preparation containing Tulsi extract significantly shorten the course of illness, clinical symptoms & biochemical parameters, in patients with viral hepatitis viral encephalitis.

(***ii). Anti malarial effect[76]:*** Essential oil of Tulsi has been reported to possess 100% larvicidal activity against the culex mosquito. Trails have shown excellent anti malarial activity of Tulsi. Its extracts have marked insecticidal activity against mosquito. Its repellent action lasts for about two hours.

(iii). Antistress / Adaptogenic effect[77,78]: Extracts from the plant have been found to reduce stress.

(iv). Anti diabetic effect [79,80]: A randomized placebo - controlled cross-over single blind trial on 40 human volunteers suffering from Type-II diabetes was performed. During the four week trials, subjects alternately received a daily dose of 2.5 gm Tulsi leaves powder or a placebo for two week period. The results showed 17.6% reduction in fasting blood glycose & 7.3% decline in PPBS on treatment with Tulsi as compared to the blood glucose levels treatment with placebo.

(vii).Other effect [81-82]: The leaves in the form of a paste are used in parasitical diseases of the skin and also applied to the finger the toe nails during fever when the limbs are cold. The juice of the leaves is given in catarrhs & bronchitis in children. The plant is said to have carminative diaphoretic & stimulant properties. A decoction of

the plant is used for cough & also as mouthwash for relieving toothache, it is good for headache, convulsions, cramps, fever & cholera.

CONCLUSION

The nutritional and pharmacological properties of the whole herb in its natural form, as it has been traditionally used, may results from synergistic interactions of many different active phytochemicals. Consequently, the overall effects of Tulsi cannot be fully duplicated with isolated compounds or extracts. Because of its inherent botanical and biochemical complexity, Tulsi standardization has, so far, eluded modern science. Although, Tulsi is known as a general vitalizer and increase physical endurance, it contains no caffeine or other stimulant. . A significant complementary role is emerging for traditional herbal medicines and holistic approaches to health in the prevention and treatment of the passive illness of modern civilization. Pharmacognostic evaluation of Tulsi helps in standardization of the plant which is the need of thehour.

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