

Review Article

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## A Review on Phytopharmacological Perceptive of *Ocimum sanctum*

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### ABSTRACT

The medicinal plants are widely used by the traditional medical practitioners for curing various diseases in their day-to-day practice. In traditional systems of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of *Ocimum sanctum* Linn (Tulsi), a small herb seen throughout India, have been recommended for the treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, insect bite etc... The phytochemical analysis signifies the presence of polyphenols, flavonoids, terpenoids, amino acids, unsaturated fatty acids, and essential elements (vitamins and minerals) which is responsible for different pharmacological activities such as anticancer, antioxidant, antimicrobial, anti-inflammation, etc. The present review summarizes the information concerning pharmacological activities such as anticancer, antioxidant, anti-inflammatory, antipyretic, antiulcer, analgesic, radiation protective, antihyperlipidemic, antidiabetic, antistress, anticataract, anticoagulant, anticonvulsant, genoprotective, hepatoprotective, neuroprotective, antimicrobial, antiarthritic, antiasthmatic, mosquitocidal and antiplasmodial activity. Diversified phenolic and flavonoid phytoconstituents of tulsi are responsible for anti-oxidant, antimicrobial, hypolipidemic, hepatoprotective, neuroprotective, antistress, antidiabetic, antiulcer, anticancer, anti-inflammatory, etc. Further, therapeutic potency of tulsi reflects in clinical trial reports provide satisfactory results with negligible adverse effects, which might be emerge as a green medicine to lessen the global burden of microbial, inflammation, metabolic associated disorders, etc.

#### Keywords

*Ocimum sanctum*,  
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### Introduction

The genus *Ocimum* belongs to the family Lamiaceae, comprises about 68 species indigenous to tropical regions of Asia, Africa

and central and south America (ThePlantList, 2013). *Ocimum sanctum* Linn. (*O. sanctum*) synonym *Ocimum tenuiflorum* L. (Lamiaceae), the *O. sanctum* prominent species of the genera is cultivated worldwide for its medicinal, perfumery, religious,

ceremonial, food and essential oil importance. *O. sanctum* is a short-lived perennial shrub of 30–60 cm height with hairy stems and sparsely hairy leaves, which distributed in the Himalayas up to an altitude of 6000 feet. Traditional uses and ayurvedic recommendations *O. sanctum*, known as Tulsi has been described as Rasayana drug in the ancient texts of Ayurveda including Charak Samhita, Susrut Samhita and Rigveda (3500–1600 BCE) to treat cough, respiratory disorders, poisoning, impotence and arthritis (Sangeetha and Poornamathy, 2015). Tulsi is identified as two common cultivars, Rama Tulsi with green leaves and Krishna Tulsi with purple leaves. *O. sanctum* have been reported for antidiabetic, wound healing, antioxidant, radiation protective, immunomodulatory, antifertility, anti-inflammatory, anti-microbial, antistress and anticancer activities.

The toxicity studies suggest that *O. sanctum* is a nontoxic herb and safe to human use (Gautum and Goel, 2014). The essential oil is one of the chemosystematic features of *O. sanctum* and a good natural source of eugenol. *O. sanctum* essential oil has commercial importance in various industries including pharmaceutical, cosmetics and food as an antiallergic and antimicrobial agent (Kumar *et al.*, 2010).

*O. sanctum* contains vitamin A, vitamin C,  $\beta$ -carotene, chlorophyll, insoluble oxalates, protein (30 Kcal), fat (0.5 g), carbohydrate (2.3 g), minerals and other phytonutrients. Each 100 g of leaf contain vitamin C (83  $\mu$ g), carotene (2.5  $\mu$ g), Ca (3.15%), P (0.34%), Cr (2.9  $\mu$ g), Cu (0.4  $\mu$ g), Zn (0.15  $\mu$ g), V (0.54  $\mu$ g), Fe (2.32  $\mu$ g) and Ni (0.73  $\mu$ g) (Pattanayak *et al.*, 2010). The antioxidant contents in *O. sanctum* leaves were found to be total carotenoid content ( $19.77 \pm 0.01$  g/100 g), total phenolic content ( $2.09 \pm 0.10$  g/100 g) and total flavonoid content ( $1.87 \pm$

0.02 g/100 g) of dry weights. The presence of ascorbic acid (8.21 mg/100 g), riboflavin (0.06 mg/100 g) and thiamine (0.3 mg/100 g) contents further suggest that *O. sanctum* leaves can intake as a dietary supplement, an alternative economic source of vitamins and natural antioxidant. Basil seed gum or mucilage is composed of two major components (i) an acid stable core glucomannan and (ii)  $\alpha$ -linked xylan including acidic side chains at C-2 and C-3 of xylosyl residues in acid-soluble portion (Naji-Tabasi *et al.*, 2016). The seed mucilage of *O. sanctum* (yield  $\sim$ 30%), is a natural polymer that contains hexouronic acid (27.25%), pentoses (38.9%) and ash (0.2%) (Khare, 2016). *O. sanctum* seed mucilage has shown protein and amino acids on phytochemical evaluation, and possess swelling index 20 ml (water) with low ash value (Kadam *et al.*, 2012). These physicochemical properties of mucilage direct towards its pharmaceutical excipient potential.

The present review on *O. sanctum* aims to provide phytochemical and pharmacological perspective aspects on its traditional uses, chemical constituents, nutritional values and pharmacological activities. This study on phytochemical constituents and their reported pharmacological activities will serve as a chemical database for future research as well as enable to understand the research gap and outlook for future *O. Sanctum*.

### **Chemical Constituents of *O. sanctum***

*O. sanctum* leaves are rich in volatile oil (0.7%), phenolics, flavonoids, neolignans, terpenoids and fatty acid derivatives. *O. sanctum* seeds contain fixed oil (18–22%), mucilage, polysaccharides and  $\beta$ -sitO. Sanctumterol in the unsaponifiable matter. *O. sanctum* seed oil is rich in triglycerides (94–98%) in which linolenic acid (43.8%) is the main content (Naji-Tabasi *et al.*, 2016).

## Phenolics

The total phenolic content in *O. sanctum* leaves has been found  $4.07 \pm 0.11$  g gallic acid equivalent/100 g dry weight (Koroch *et al.*, 2010). Caffeic acid, chlorogenic acid, vanillic acid, ocimum naphthanoic acid and menthylsalicylic glucO. Sanctumide were isolated from the aerial parts of *O. sanctum* (Ali and Ali, 2012). 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-hydroxybenzaldehyde were confirmed by HPLC using authentic samples. Rosmarinic acid, an ester of caffeic acid is quantified as 0.27% w/w in *O. sanctum* leaves using APCI mass spectrometry technique (Sundaram *et al.*, 2012).

## Flavonoids

Flavonoids are the major class including methoxy flavonoids and their glycosides (luteolin, isothymusin, cirsimartin), C-glycO. sanctumides flavonoids (orientin, isoorientin, isovitexin and vicenin) from *O. sanctum*.

Grayer *et al.*, (2001) studied the distribution of 8-oxygenated flavones on *O. sanctum* leaf surface using atmospheric pressure chemical ionization mass spectrometry (APCI-MS) and identified apigenin, cirsimartin, salvigenin, crisilineol, eupatorin, isothymusin and gardenin.

The analysis shows that flavone-7-O-glycO. sanctumides are the characteristics of *O. sanctum*, whereas luteolin-5-O-glycO. sanctumide considered as the marker compound in all nine species of Ocimum, including *O. sanctum* (Grayer *et al.*, 2002). The flavones apigenin, isothymusin, cirsimartin and crisilineol were isolated from the aerial parts of *O. sanctum*.

## Phenyl propanoids

Eugenol is one of the most distributed phenyl propanoid in the essential oil of *O. sanctum* leaves. Other phenyl propane derivatives such as ociglycoside or eugenyl- $\beta$ -D-glucoside, citrusin C, ferulaldehyde, bieugenol and dehydrodieugenol were isolated from the leaves of *O. sanctum* (Suzuki *et al.*, 2009).

## Neolignans

The methanol extract of *O. sanctum* leaves revealed seven novel neolignans named as Tulsinol A to Tulsinol G (Suzuki *et al.*, 2009). These neolignans are formed by the polymerization of eugenol.

## Coumarins

Three coumarins named ocimarin, aeculetin and aesculin were reported from *O. sanctum*. (Gupta *et al.*, 2007).

## Terpenoids

Different terpenoids like sesquiterpenoids ( $\beta$ -caryophyllene and 4,5-epoxy-caryophyllene), abietane diterpenoid (carnosic acid), olean triterpenoids (oleanolic acid,  $\beta$ -Amyrin-glucopyranoside) and ursane triterpenoids have been reported from *O. sanctum* (Suzuki *et al.*, 2009; Baliga *et al.*, 2013). The quantification studies revealed ursolic acid as the most abundant constituent in *O. sanctum* with 0.252%–0.478% w/w and 0.62–19.10 mg/g using HPTLC and UPLC-ESI-MS/MS, respectively (Pandey *et al.*, 2014). Two separate antidiabetic activity-guided isolation on *O. sanctum* roots and aerial parts provided two novel triterpenoids named urs-12-en-3 $\beta$ ,6 $\beta$ ,20 $\beta$ -triol-28oic acid and 16-hydroxy-4,4,10,13- tetramethyl-17-(4-methyl-pentyl)-hexadecahydrocyclopenta [ $\alpha$ ] phenanthren-3-one, respectively (Ahmad *et al.*, 2012a). Further, a new tricyclic sesquiterpenoid 2-

(hydroxymethyl)-5,5,9-trimethylcycloundecan-2-ol along with  $\beta$ -caryophyllene, elemene,  $\alpha$ -humulene,  $\alpha$ -caryophyllene, germacrene-A, trans- $\alpha$ -bergamotene and 5 $\beta$ -hydroxycaryophyllene were isolated from *O. sanctum* leaves. The novel tricyclic sesquiterpenoid was biosynthetically derived from  $\beta$ -caryophyllene (Singh *et al.*, 2014).

### **Steroids**

Four commonly occurring phytosterols  $\beta$ -sitosterol,  $\beta$ -sitosterol-3-O $\beta$ -D-glucopyranoside, stigmasterol and campesterol were isolated from leaves and stems of *O. sanctum* (Baliga *et al.*, 2013).

### **Essential oil**

*O. sanctum* essential oil (yield 0.3-4.1%) is mainly composed of terpenoids including acyclic monoterpenoids, monocyclic terpenoids, bicyclic terpenoids, aliphatic aldehydes, phenolic acids, esters and sesquiterpenoids. The composition and yield of *O. sanctum* essential oil are differed with harvesting at different localities, cultivars (green and purple), collection periods, stages of harvesting and climatic conditions (Saharkhiz *et al.*, 2015). Eugenol or methyl eugenol and/or methyl chavicol were found as the major constituents of *O. sanctum* essential oil by considering the different harvesting stages and cultivars (Mondello *et al.*, 2002). The major diversities in *Ocimum* species were found in Africa followed by South America (Brazil) and Asia (India). Eugenol (27–83%) was found as the main component of oils from USA, India, Germany, Thailand, Cuba and Brazil, whereas oil from plants grown in Australia contain mainly methyl chavicol (87%) (Vani *et al.*, 2009). More interestingly, the decreasing concentration of eugenol and methyleugenol contents in *O. sanctum* essential oil in matured leaves might be their involvement in polymerization and

synthesis of neolignans (Suzuki *et al.*, 2009), and/or further oxidation of phenolic compounds catalyzed by the increase of polyphenoxidase and peroxidase activity (Dey and Choudhuri, 1983). The aroma compounds of *O. sanctum* essential oil (methyl eugenol chemotype, 56.18%) were identified by solid phase microextraction (SPME)/GC–MS/flame ionization detection (FID) and olfactive evaluations. The spicy-green-notes of *O. sanctum* essential oil is due to methyl eugenol,  $\beta$ -caryophyllene,  $\beta$ -caryophyllene oxide and germacrene D, while spicy-peppery-notes corresponds to germacrene D (Jirovetz *et al.*, 2003). Moreover, the major pharmacological activities of *O. sanctum* essential oil such as mosquitocidal, antimicrobial and anthelmintic were found due to its marker constituent eugenol (Kumar *et al.*, 2010). *O. sanctum* essential oil (40  $\mu$ g/ml) was found to be non-toxic to the mammalian kidney fibroblast (VERO) and kidney epithelial cells (LLC-PK11) using Neutral Red assay (Zheliakov *et al.*, 2008).

### **Fixed oil (non-volatile oil)**

The fixed oil content in *O. sanctum* seeds was found ~18-22% and, composed of mainly linoleic acid (66.1%),  $\alpha$ -linolenic acid (15.7%), oleic acid (9.0%), palmitic acid (6.94%) and stearic acid (2.1%). The major components of fixed oil, linoleic acid and linolenic acid (an  $\omega$ -3 fatty acid, cis9,12,15-octadecatrienoic acid) were supposed to be responsible for its anti-inflammatory, anticoagulant, hypotensive, chemopreventive, antihypercholesterolaemic and immunomodulatory activities (Singh *et al.*, 2007). Fixed oil of *O. sanctum* is reported for anti-inflammatory, antiarthritic, antimicrobial and antiulcer properties (Singh *et al.*, 2001; Singh *et al.*, 2005). The anti-inflammatory activity of fixed oil is due to the dual inhibition of arachidonate metabolism and antihistaminic activity (Singh *et al.*, 2007). Only one report

is available on the isolation of fixed oil (yield 1.046%) from *O. sanctum* leaves, along with the antidiabetic and antioxidant potential. The fixed oil extracted from *O. sanctum* leaves was rich in  $\alpha$ -linolenic acid (60.60%), linoleic acid (17.86%) and palmitic acid (15.65%) (Suanarunsawat *et al.*, 2016).

### **Fatty acid derivatives**

Fatty acid derivatives were isolated from the leaves and roots of *O. sanctum*, including four cerebrosides (Ahmad *et al.*, 2012). Fatty acid derivatives like palmityl glucoside and sanctumoic acid were exhibited mosquitocidal activity, while cerebrosides showed antistress activity (Kelm and Nair, 1998).

### **Polysaccharide**

A polysaccharide (~106 Da) isolated from *O. sanctum* leaves contain monosaccharide compositions rhamnose (23.3%), xylose (19.2%), arabinose (42.2%), glucose (10.3%) and galactose (5.0%) (Subramanian *et al.*, 2005).

### **Other secondary metabolites**

An acetone oligomer named (E)-6-hydroxy-4,6-dimethyl-3-heptene-2-one was isolated as a colorless oil from the aerial parts of *O. sanctum* (Kelm and Nair, 1998).

### **Pharmacological Activities of *O. sanctum* Secondary Metabolites**

The chemical constituents of *O. sanctum* are mainly studied for its therapeutic potential like anticancer, antioxidant, anti-inflammatory, anti-pyretic, antiulcer, analgesic, radiation protective, antihyperlipidemic, antidiabetic, antistress, anticataract, anticoagulant, anticonvulsant, genoprotective, hepatoprotective, neuroprotective, leishmanicidal, antimicrobial, antihelminthics, antiarthritic activity,

antihistamine activity, mosquitocidal and antiplasmodial activity.

### **Anticancer activity**

A tricyclic sesquiterpenoids isolated from the oil of *O. sanctum* leaves showed antiproliferative activity against MCF-7 cell line using doxorubicin as standard (Singh *et al.*, 2014). In continuation of antiproliferative screening against MCF-7 cell line, sesquiterpenes  $\beta$ -car-yophyllene, 4,5-epoxycaryophyllene and 5 $\beta$ -hydroxycaryophyllene showed, respectively. Compounds *O. sanctum*marinic acid, apigenin, luteolin, orientin, vicenin-2, ursolic acid andoleanolic acid are well studied for their anticancer potential (Nagaprashantha *et al.*, 2011). Banerjee *et al.*, reported anticancer activity of *O. sanctum* against many carcinogenic agents. Juice of fresh leaves of *O. sanctum* has anticancer property in cancer subjects. Alcoholic extracts of *O. sanctum* act on the activities of cytochrome P-450, cytochrome b5 and aryl hydrocarbon hydroxylase in liver and glutathione-S-transferase (GST) and a reduced glutathione level in liver, lung. All these enzymes and cofactors play an important role in the detoxification of carcinogens and mutagens. *O. sanctum* leaves when fed to experimental rats for ten weeks, significantly reduced the squamous cell carcinoma and hematoma incidences. The terpenoids and flavonoids are the major class of compounds responsible for the anticancer activity of *O. sanctum*.

### **Antioxidant activity**

Phenolics/flavonoids of *O. sanctum* were investigated for their free radical scavenging activity. The antioxidant activity guided isolation of *O. sanctum* leaves and stems in lipophilic *O. sanctum* oxidation model yielded six flavonoids including apigenin, *O. sanctum* marinic acid, isothymusin, isothymonin,

cirsimaritin, cirsilineol along with eugenol (Kelm *et al.*, 1998). Compounds isothymusin, isothymonin and eugenol showed good antioxidant activity at 10  $\mu$ M, compared to standards TBHQ (terbutyl hydroquinone) and BHT (butylated hydroxyl toluene). Koroch *et al.*, (2010) found *O. sanctum* marinic acid as the main constituent responsible for the antioxidant activity of *O. sanctum* due to its rapid scavenging effect of free radicals. The polysaccharide showed potent DPPH free radicals scavenging activity with IC<sub>0.2</sub> value of  $5.61 \pm 0.17$   $\mu$ g/ml, compared to  $\alpha$ -tocopherol and BHA. Also, *O. sanctum* polysaccharide scavenged ~ 54% and ~79% of superoxide free radicals at 10 and 50  $\mu$ g/ml, respectively. The antioxidant results showed that *O. sanctum* processes reactive oxygen species scavenging and iron chelating properties. The pretreatment of *O. sanctum* polysaccharide at 100  $\mu$ g/ml protects  $30 \pm 3.2\%$  mouse splenocytes against  $\gamma$ -ray irradiation. The antioxidant potential of *O. sanctum* polysaccharide against oxidative damage to lipid, DNA and splenocytes warrants its application in radiation protection. The aqueous, hydroalcoholic and methanolic extracts of *O. sanctum* show significant antioxidant activity, both *In vivo* and *In vitro*. Phytochemical investigations of *O. sanctum* leaf extract show phenols and flavonoids. Oral feeding of OS provides significant liver and aortic tissue protection from hypercholesterolemia induced peroxidative damage.

### **Anti-inflammatory activity**

Eugenol was the *O. sanctum* active compound and showed 97% of COX-1 inhibition at 1000  $\mu$ M, compared to ibuprofen, naproxen and aspirin with 33%, 58% and 46% COX-1 inhibition at 10, 10 and 1000  $\mu$ M, respectively (Kelm *et al.*, 1998). Moreover, cirsineol, cirsimaritin, isothymonin and apigenin showed 37%, 50%, 37% and 65% COX-1

enzyme inhibition, respectively. *O. sanctum* fixed oil exhibited anti-inflammatory effect due to the dual inhibition of arachidonate metabolism and antihistaminic activity (Singh *et al.*, 2007). The fresh tulsi leaf in its paste form showed anti-inflammatory activity using carrageenan induced paw edema model in comparison to Indomethacin. The percent inhibition of 500 mg/kg of the tulsi paste was found to be 88.15% as that of the response observed with 100 mg/kg of indomethacin and showed considerable anti-inflammatory activity. The results suggest that linolenic acid present in *O. sanctum* fixed oil has the capacity to block both the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism and could be responsible for the anti-inflammatory activity of the oil.

### **Antipyretic activity**

The antipyretic activity of *O. Sanctum* fixed oil was evaluated by testing it against typhoid paratyphoid A/B vaccine-induced pyrexia in rats. The oil on ip administration considerably reduced the febrile response indicating its antipyretic activity. The antipyretic activity of the oil was comparable to aspirin. Further, the fixed oil *O. Sanctum* possessed prostaglandin inhibitory activity and the same could explain its antipyretic activity (Godhwani, 1987).

### **Antiulcer activity**

The aqueous extract of *O. sanctum* (100 mg/kg and 200 mg/kg orally) exhibited significant protection against ethanol induced gastric ulceration in Wistar rats. *O. sanctum* exhibits antiulcer activity by enhancing antioxidant potential of gastric mucosa. It was found that the ethanolic extract of *O. sanctum* not only reduced acid secretion, but also potentially elevated the mucoprotective effect and 100 mg/kg body weight was found to be exhibit antiulcerogenic in all the five models against ulcer induced by cold restraint (CRU),

alcohol (AL), aspirin (ASP), and pyloric ligation (PL) model in rats, and histamine (HST) induced duodenal ulcer model in guinea pigs 32. The fixed oil of *O. Sanctum* administered i.p. shows significant antiulcer activity against aspirin, indomethacin, alcohol (ethanol 50%), histamine, reserpine, serotonin or stress induced ulcers in rats. The fixed oil significantly *O. sanctum* possessed antiulcer activity due to its lipoxygenase inhibitory, histamine antagonistic and antisecretory effects (Singh and Majumdar, 1999).

### **Analgesic activity**

Tulsi showed an increase of 20.34 % with mild, 43.80 % with moderate *O. sanctum* and of 51.47 % with maximum *O. sanctum* at 90 min. after injection. The regression line indicated that the analgesic effect remains upto 3 hours irrespective of *O. sanctum* concentration. Analysis of variance revealed that the analgesic activity of Tulsi was statistically significant with all the three *O. sanctum* concentrations. The analgesic activity of fixed oil from the seeds of *O. sanctum* were investigated in mice and rats using the tail flick, tail clip, tail immersion and acetic acid-induced writhing methods. It was found it be effective against acetic acid induced writhing in dependent manner, suggesting that writhing inhibiting activity of the oil is peripherally mediated due to combined inhibitory effects of prostaglandins, histamine and acetylcholine (Singh and Majumdar, 1995).

### **Radiation protective activity**

The optimum *O. sanctum* extract for radiation protection was found to be 50 mg/kg b.w.(i.p.), with LD 50 6.0 g/kg body weight. Further, chemical investigation on *O. sanctum* aqueous extract gave two water soluble flavonoids orientin and vicenin. Both, the flavonoids exhibited protective effect against

radiation-induced chromosomal damage in mice due to their free radical scavenging and metal chelating effects. The iron chelating effect of flavonoids inhibited the formation of thiobarbituric acid reactive substances (TBRAS) that protects lipid peroxidation initiated by iron ion bound to the lipid membrane. The low and non-toxic concentration of orientin and vicenin showed significant radiation protection to human peripheral lymphocytes (*in-vitro*), suggest their clinical application in cancer radiotherapy as normal tissues protector (Vrinda and Devi, 2001). In 2011 studied the radioprotective effect of *O. sanctum* on the salivary gland of rats administered radioiodine and compared its efficacy with a known radioprotectant and supplemented and subsequently exposed to rats at 3- and 6-months duration exhibited comparable histopathology with control and indicated *O. sanctum* radioprotective effect. Flavonoids extracted from the leaves of, *O. sanctum* were studied as a radioprotector on the erythrocyte antioxidants in oral cancer.

### **Antihyperlipidemic and antidiabetic activity**

*O. sanctum* leaves have been studied for serum lipid lowering activity in both normal albino rabbits and diabetic rats, the antihyperlipidemic effect is mainly due to its essential oil content (Suanarunsawat *et al.*, 2016). *O. sanctum* essential oil riched with eugenol (18.25%), methyl eugenol (47.06%) and  $\beta$ -caryophyllene (23.68%) has been reported to suppress the serum total cholesterol ( $93.62 \pm 3.29$ ) mg/dl and triglycerides ( $36.29 \pm 3.33$  mg/dl) in hypercholesterolaemic rats, compared to the negative control i.e. high cholesterol treated rats (total cholesterol,  $138.12 \pm 10.21$  mg/dl and triglyceride,  $50.79 \pm 2.86$  mg/dl). *O. sanctum* essential oil also showed antihyperlipidemic effects comparable with

standard drug simvastatin (total cholesterol,  $90.35 \pm 5.70$  mg/dl and triglyceride,  $48.50 \pm 4.35$  mg/dl). The antihyperlipidemic activity of *O. Sanctum* essential oil was due to the suppression of liver lipid synthesis, and the presence of phenylpropanoid constituents. These antihyperlipidemic results suggest that *O. sanctum* essential oil is potentially beneficial in the prevention and treatment of diseases like atherosclerosis *O. sanctum* and cardiovascular disorders (Suanarunsawat *et al.*, 2009). The fixed oil obtained from fresh *O. sanctum* leaves constitute of mainly  $\alpha$ -linolenic acid (60.60%), which significantly lower the diabetically elevated blood glucO. sanctume levels and serum lipid profile with an increase in serum insulin levels in streptozotocin-induced type 1 diabetes mellitus rats within three weeks (Suanarunsawat *et al.*, 2016). Additionally, fixed oil suppresses the elevated TBRAS level and increases the activity of antioxidative enzymes in the liver and cardiac tissues. The bioactive fraction (20 mg/kg) exhibited significant ( $p < 0.001$ ) anti-diabetic activity and decreases the level of serum glucose, triglycerides, LDL cholesterol and total cholesterol in alloxan induced diabetic rats.

### **Antistress activity**

*O. sanctum* is well known for its adaptogenic and immunomodulatory properties since ancient time and these potentials credited to its antistress activity. The extract obtained by the blending of water and methanol extracts of *O. sanctum* whole plant with the required level of active constituents, ociglycoside-I ( $> 0.1\%$  w/w), rosmarinic acid ( $> 0.2\%$  w/w), oleanolic acid and ursolic acid ( $> 2.5\%$ ), which was found to be effective against chronic variable stress (Richard *et al.*, 2016).

The antistress effects were studied on cortisol release and CHHR1 receptor activity using

cell-based assay, while  $11\beta$ -hydroxysteroid dehydrogenase type-1 ( $11\beta$ -HSD1) and catechol-O-methyltransferase (COMT) for cell-free assays. Further, the extract showed inhibitory activity on COMT ( $IC_{50} = 11.65$   $\mu$ g/ml) and  $11\beta$ -HSD1 (99.96% at 200  $\mu$ g/ml) compared to the standard 3,5-dini-trocatechol ( $IC_{50} = 24.91$  nM) and carbenoxolone (61.44% at 600 nM). Moreover, *O. sanctum* (6.25–100  $\mu$ g/ml) also inhibits cortisol release in forskolin-induced human adreno-carcinoma cells (NCI-H295R) and this effect might be attributed by ursolic acid (625  $\mu$ M to 10  $\mu$ M).

Thus, inhibition of cortisol release, blocking the CRHR1 receptor, inhibition of  $11\beta$ -HSD1 and COMT effects are found to be responsible for the antistress activity of *O. sanctum*. In a separate study, the ethanol extract of *O. sanctum* leaves and its n-butanol fraction significantly ( $p < 0.05$ ) normalize the acute stress and chronic predictable stress at a dose of 200 mg/kg body weight, compared to the standard drug Panax quinquifolium at 100 mg/kg bodyweight (Gupta *et al.*, 2007). The prior treatment of all three compounds significantly reduces the increased cortisone levels ( $p < 0.05$ ) and creatine kinase levels ( $p < 0.01$ ) at 40 mg/kg body weight in acute stress induced rats, compared to the normal group. Fresh leaves of *O. sanctum* were evaluated for antistress activity against experimentally induced oxidative stress in albino rabbits (Jyoti *et al.*, 2007).

### **Anticataract activity**

The Aqueous extract of fresh leaves of *O. sanctum* (1g/kg and 2 g/kg) significantly delayed the onset as well as subsequent maturation of cataract in galactosaemic cataract model in rats by 30% galactose and naphthalene cataract model in rabbits by 1 g/kg naphthalene (Gupta *et al.*, 2002).



### **Anticoagulant activity**

*O. sanctum* fixed oil (3 ml/kg, ip) was studied for anticoagulant activity. It was observed that blood clotting time was prolonged and the response was comparable to that obtained with aspirin (100 mg/kg). The effect appears to be due to the antiaggregatory action of oil on platelets (Singh *et al.*, 2001).

### **Anticonvulsant activity**

Different extractives of stem, leaf and stem callus of *O. sanctum* were tested for anticonvulsant activity against standard drug phenytoin using maximal electro shock (MES) model. Ethanol and chloroform extractives of stem, leaf and stem calli were effective in preventing tonic convulsions induced by transcorneal electro shock (Jaggi *et al.*, 2003).

### **Genoprotective activity**

Protective effect of galactosaemic was evaluated on chlorpyrifos-induced genotoxicity in *In vivo* and *In vitro* models. It was observed that rats pretreated with *O. Sanctum* extract, showed a significant ( $P < 0.01$ ) increase in mitotic index a significant decrease in the frequency of aberrant cells as compared to the rats treated with chlorpyrifos alone. A significant ( $P < 0.05$ ) increase in chromosomal aberrations was observed in cultures treated with 75  $\mu\text{g/ml}$  chlorpyrifos as compared to controls, which decreased significantly ( $P < 0.05$ ) with *Ocimum sanctum* extract pretreatment (Khanna *et al.*, 2011).

### **Hepatoprotective activity**

The hepatoprotective activity of *O. sanctum* leaf extract was studied against paracetamol-induced liver damage in Albino rats synergism with silymarin and concluded that

*Ocimum sanctum* alcoholic leaf extract showed significant hepatoprotective activity and synergism with silymarin (Lahon and Das, 2011). When alcoholic extract of Tulsi plant orally administered, it exhibited hepatoprotective effect against Paracetamol, Carbontetrachloride and anti-tuberculosis drugs induced liver injury in albino rats. When extract of *O. sanctum* was used in male albino rats weighing 100-150 g of Wistar strain (5-6weeks) the level of enzymes was reduced.

### **Neuroprotective activity**

*O. sanctum* shows ameliorative potential in attenuating vincristine induced peripheral neuropathic pain in rats, which may be attributed to decrease in oxidative stress and calcium levels. Administration of *O. sanctum* (100 and 200 mg/kg p.o.) and its saponin rich fraction (100 and 200 mg/kg p.o.) for 14 days significantly attenuated vincristine-induced neuropathic pain along with decrease in oxidative stress and calcium levels (Kaur *et al.*, 2010).

### **Leishmanicidal activity**

The essential oil from *O. sanctum* exhibited leishmanicidal activity against *Leishmania donovani* (Zheliakov *et al.*, 2008). The essential oil demonstrated leishmanicidal activity with  $\text{IC}_{50} = 37.3 \pm 4.6 \mu\text{g/ml}$  and  $\text{IC}_{90} = 90.0 \pm 4.6 \mu\text{g/ml}$ , using pentamidine and amphotericin Bas positive controls. Interestingly, the major contents of essential oil eugenol and methylehavicol did not possess leishmanicidal activity, while minor constituent (+)- $\delta$ -cadinene (yield  $0.168 \pm 0.0194\%$ ) showed potent leishmanicidal activity ( $\text{IC}_{50} = 4.0 \mu\text{g/ml}$  and  $\text{IC}_{90} = 7.0 \mu\text{g/ml}$ ). The hydroalcoholic extract of *O. sanctum* leaves inhibits the growth of promastigotes of *L. amazonensis* by  $8.8 \pm 1.2\%$  at 50  $\mu\text{g/ml}$  and  $10.3 \pm 1.3\%$  at 100

µg/ml, compared to pentamidine  $96.9 \pm 0.2\%$  and  $99.2 \pm 0.3\%$  at 50 and 100 µg/ml (Garcia *et al.*, 2010). The leishmanicidal activity guided isolation of *O. sanctum* ethyl acetate fraction resulted ferulaldehyde and ursolic acid with IC<sub>50</sub> values 0.9 µg/ml and 2.2 µg/ml, respectively against promastigotes of major compared to positive control amphotericin B (IC<sub>50</sub> = 0.04 µg/ml) (Suzuki *et al.*, 2009). Eugenol and caryophyllene oxide showed IC 50 values > 25 µg/ml against *L. major*, while eugenol dimers bieugenol (IC<sub>50</sub> = 13.6 µg/ml) and dehydrodieugenol (IC 50 = 16.9 µg/ml) were found better leishmanicidal components. Also, a novel neolignan tulsinol C exhibited potent leishmanicidal activity with IC<sub>50</sub> value 9.1 µg/ml against *L. major* (Suzuki *et al.*, 2009).

### **Antimicrobial activity**

*O. sanctum* flavonoids orientin and vicenin were screened against bacterial strains causing urinary tract infection in human e.g. *Staphylococcus aureus*, *Staphylococcus cohnii*, and *Escherichia coli*, *Proteus* and *Klebsiella pneumonia* (gram negative) using disc diffusion method (Ali and Dixit, 2012). Orientin (400 mg/ml) showed antibacterial activity against *S. aureus*, *S. cohnii* and *K. pneumonia* with maximum zone inhibition (ZOI) of 18.04, 17.13 and 16.11 mm, respectively. While, vicenin at 400 mg/ml was found to be active against *E. coli* (ZOI, 18.84 mm) and *Proteus* (ZOI, 17.16 mm).

Moreover, the synergistic effect of orientin and vicenin (in a ratio of 1:1) on antibacterial activity showed better results in all the strains than individual flavonoids with maximum ZOI of 20.12, 20.75, 20.95 and 20.31 mm at 400 mg/ml concentrations against *E. coli*, *Proteus*, *S. aureus*, *S. cohnii* and *K. pneumonia*, respectively. The antibacterial activity results were concluded that the potent

synergistic effect of flavonoids orientin and vicenin can be used as a new choice for the treatment of bacterial infected UTI infections.

Antimicrobial activity of different extracts (Ethanol, Methanol, Ethyl acetate and chloroform) of dried leaf of *O. sanctum* were tested against three human pathogens strains such as *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* through the well diffusion and the poison plate method. The Minimum inhibitory concentration (MIC) values of the crude extract of the tested plant leaves were determined. Both methods (well diffusion and poison plate) showed the strongest activity in methanol extract. Among four methanol extracts, they show more inhibition against in *S. aureus* than *E. coli* and *C. albicans*. The antimicrobial activity of *O. sanctum* leaf extract in normal tap water and local river water was investigated.

The antimicrobial effect was studied with different concentration (100 to 600 mg l<sup>-1</sup>) of Tulsi leaf extract in tap and river water. In this, 600 mg l<sup>-1</sup> concentration of plant extract treated water showed effective antimicrobial activity at 15 to 16 hrs than the other concentration of extract. The 500 mg l<sup>-1</sup> of extract treated water showed 95 to 98% antibacterial activity in 14 to 16 hrs. The minimum bacterial concentration (MBC) was observed in 500 and 600 mg l<sup>-1</sup> extract concentration. The concentration of the bacterial cells inhibited gradually for an hour was studied by spread plate method (Kayastha, 2014).

Similarly, Geeta *et al.*, reported that on comparing alcoholic and aqueous extract, the aqueous extract of *O. sanctum* (60 mg/kg) showed wide zones of inhibition against *Klebsiella*. With fresh juice and honey, worms and parasites are removed; the sweetness excites the parasites out. It is used in the treatment of viral encephalitis, malaria

and typhoid. Methyl chavicol and linalool obtained from essential oil of *O. sanctum* showed significant antifungal activity against *Candida*, including a zole-resistant strains. Their fungicidal action resulted from extensive lesions of the plasma membrane and a considerable reduction in the amount of ergosterol *O. sanctum*. Antifungal activity of *O. sanctum* leaves was determined against clinically isolated dermatophytes.

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of various extracts and fractions of *O. sanctum* leaves were also determined against dermatophytic fungi used (Khan *et al.*, 2010). Different types of extracts of *O. sanctum* have anti-viral activity against different viruses e.g. Hematopoietic *O. sanctum* is Virus (IHNV), polio virus type 356, herpes virus (HSV), hepatitis B virus, new castle Disease Virus. Ethanolic extract of Tulsi plant leaves in a range of 22.5 mg/ml concentration inhibit replication of polio type 3 virus in VERO cells. The extracted components of this plant like linalool, apigenin and ursolic acid show broad spectrum antiviral activity against DNA viruses like RNA virus and adenoviruses. One study also proves its efficacy against new castle disease of poultry (Jayati *et al.*, 2013). The essential oil of *O. sanctum* and eugenol, tested *In vitro*, showed potent anthelmintic activity in the *Caenorhabditis elegans* model (Asha *et al.*, 2001).

### **Antiarthritic Activity**

The fixed oil of *O. sanctum* seeds was screened for antiarthritic activity by Singh *et al.*, 2007 using Freund's adjuvant arthritis, formaldehyde-induced arthritis and turpentine oil induced joint edema in rats. The fixed oil showed significant anti-arthritic activity in both models and anti-edema activity against turpentine oil-induced joint edema (Singh and Majumdar, 1996).

### **Antiasthmatic activity**

50% aqueous ethanol extract of dried and fresh leaves, and the volatile and fixed oils of *O. sanctum* was evaluated against histamine and acetylcholine induced preconvulsive dyspnea (PCD) in guinea pigs. The 50% ethanol extract and volatile oil extracted from fresh leaves and fixed oil from the seeds significantly protected the guinea pigs against histamine and acetylcholine induced pre convulsive dyspnea. However, the 50% ethanol extract of dried leaves did not protect the guinea pigs against histamine induced preconvulsive dyspnea (Singh and Agrawal, 1991).

### **Mosquitocidal activity**

The *O. sanctum* moquitocidal activity against *Aedes aegyptii* larvae guided fraction of *O. sanctum* yielded two compounds eugenol and (E)-6-hydroxy-4,6-dimethyl-3-heptene-2-one (Kelm and Nair, 1998). Eugenol and (E)-6-hydroxy-4,6-dimethyl-3-heptene-2-one demonstrated *O. Sanctum* mosquitocidal activity with LD<sub>100</sub> values 200 µg/ml and 6.25 µg/ml, respectively in 24 h, while there was no mortality for control larvae. Further, the researchers suggest to investigate the different *O. sanctum* extract in search of novel *O. sanctum* mosquitocidal compounds.

### **Antiplasmodial activity**

Leaf extract, root extracts, the stem and flower extracts of *O. sanctum* showed excellent antiplasmodial activity in a study carried out by Inbaneson *et al.*, on three different species of *Ocimum*. The *in-vitro* antiplasmodial activity might be due to the presence of alkaloids, glycosides, flavonoids, phenols, saponins, triterpenoids, proteins, resins, steroids and tannins in the ethanolic extracts of tested plants (Inbaneson *et al.*, 2012).

Several chemical classes of compounds including phenolics, flavonoids, phenylpropanoids, neolignans, terpenoids, coumarins, fatty acid derivatives, essential oil and fixed oil have been reported from *O. sanctum*. The essential oil of *O. sanctum* is a good source of natural eugenol and well explored in analytical, chemical and biological aspects due to its high commercial importance in pharmaceutical, cosmetics and food industry. Fixed oil of the seeds is rich in  $\omega$ -3 fatty acids and is the recent interest of the research, due to its wide range of pharmacological properties especially in cardio protection. Flavonoids are the major class of compounds isolated from *O. sanctum* and have been found as the main active constituents. The water-soluble flavonoids, orientin and vicenin have been well explored in terms of their radiation protective effects at lower and higher dose. The hydrophilic character of both the flavonoids makes them useful for their antioxidant effect in detoxification as well as radiation protector in cancer therapy. Further studies are needed to explore their pharmacological activities and mechanism for therapeutic potential and to explore pre-clinically studied compounds for clinical practices, especially in antistress and radiation protection.

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