AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

In vitro studies of the *Ocimum sanctum:* Tulsi, Medicinal herb

Deepika Gulati[1]*, Priyanka, Minakshi Pal, Nidhi , Ikbal
1. Centre for Plant Biotechnology, CCSHAU Campus, Hisar -125001, Hariyana

ABSTRACT

In the present review on Tulsi, efforts has been done to congregate the botanical, and *in vitro* tissue culture techniques in *Ocimum sanctum* Linn., a medicinal herb used in the indigenous system of medicine. Tulsi has been adored in almost all ayurvedic literature for its adorable medicinal properties. It is pungent-bitter in taste. Its leaves are hot, light and dry while seeds are considered to be cold in effect. Several medicinal properties of Tulsi are present in the roots, leaves and seeds. It has a wide range of action on the human body mainly ain curing cough, inducer of sweat and prevents the indigestion and anorexia. Medicinal properties of Tulsi are very well known for thousand years to various parts of the world. In the Indian subcontinent, Hindus considered this medicinal herb as sacred plant. Exploration the scientific studies of traditional belief of medicinal properties of Tulsi have got acceleration after the middle of the 20th century. Most of the evidences are based upon *in-vitro,* experimental and a very few are of human studies. Till now, no review present on the in vitro studies of Tulsi, therefore, the present review would discuss the *in vitro* studies along with its botanical description. This review will definitely help the researchers who deals with Tulsi studies to know its use in proper way, herb appears to be highly valuable due to many pharmacological / medicinal properties.

Keywords: *Ocimum sanctum* Tulsi**,** Medicinal Herbs

*Corresponding Author Email: deepikahau84@gmail.com
Received 25 September 2015, Accepted 02 October 2015

Please cite this article as: Gulati D *et al*., *In vitro* studies of the *Ocimum sanctum:* Tulsi, Medicinal herb. American Journal of PharmTech Research 2015.

INTRODUCTION

In Hindu religion, Tulsi is an important symbol of the tradition. The word 'Tulsi' has another name, Vishnupriya means the one that pleases Lord Vishnu. It is found in most of the Indian homes and worshipped by all, its legend has permeated Indian ethos down the ages. Well known in English as Holy Basil and botanically called *Ocimum sanctum*, Tulsi belongs to plant family Lamiaceae. It has played an important contribution to the field of science from ancient times and same to modern research due to its vast medicinal properties. Tulsi merely needs an introduction in the talks of herbal remedies. In this critical hour when new viral strains are affecting the world population lately and people are more prone to herbal formulations; it becomes noteworthy to bring to limelight the most ancient herb which has a high reputation among medicinal plants domain. Undoubtedly, Tulsi has been an effective anti-inflammatory, antibiotic and antipyretic[1] medicine in everyday regime for cold, cough and flu. Thus, researchers across the world have extended their studies on this herb leading to the conclusions that it also possess properties like healing of wound, anti-oxidant, anti-carcinogenic, anti- inflammatory and anti-ulcerogenic.[2,3]

There are so many reports on Tulsi which bring together the various medicinal properties and extensive research findings but none was found to share the review on *in vitro* tissue culture efforts that has been made so far. Development of micro propagation protocols through plant tissue culture techniques is a significant step to create independency over the natural reservoirs. Therefore, in this article along with the newest research data, work progress in the area of plant tissue culture would be discussed.

Morphological Attributes:

Tulsi is an annual herb 35-70 cm high, branched stems usually purple in colour, sub-quadrangular, clothed with soft spreading hairs. Leaves are 2.5-5 cm. long and 1.7-3.3 cm broad, acute obstuse or oblong, elliptical, margin serrate or entire, surface pubescent on both sides, gland dotted, base acute or obtuse, 1.3-2.5 cm petioles, long slender, hairy. Flowers in present in racemes 15-20 cm forming verticillaste inflorescence. long and broad bracts with 3mm diammeter, broadly ovate; slender calyx, pubescent, bilabiate, lower lip is longer than upper lip. Corolla 4 mm long, purple in colour, bilabiate upper lip pubescent on the back. Stamens are exserted, slender filaments , the upper pair with a small appendage having a branch present at the base. Nutlets are 1.26 mm long, broadly ellipsoid, smooth, yellow with black markings. Seeds are brownish, globose or sub-globose. Aromatic odour and sharp taste present . Caeruleus fruit is present

Chemical constituents

The chemical composition of Tulsi is highly complex, containing many nutrients and biological active compounds. Standardization of the active components of Tulsi is very complicated due to its inherent biochemical complexity and botanical nature. However, best known of many active components of the Tulsi, leaves are the source of an essential oil i.e eugenol and ursolic acid[4,5]. Ursolic acid, that have been identified and extracted are one of the major constituents. It has been suggested to possess antifertility effects in male mice and rats of both sexes. Ursolic acid possess anti-estrogenic effects, causes a decrease in sperm counts[6] and reduces spermatogenesis. Eugenol is a phenolic compound and also a major constituent of essential oils extracted from different parts of Tulsi plant. The therapeutic potential of the extracted essential oils from the fresh leaves of *Ocimum sanctum L.* has been found to be largely due to major constituent i.e eugenol which itself is a phenolic compound (l-hydroxy-2-methoxy-4- allylbenzene)[7]

***In vitro* studies of Tulsi**: Tissue culture is an age-old practice for *in vitro* regeneration of plants, especially the medicinally valuable plants that are difficult to propagate in natural environment [8]This method is also used for studying the molecular studies of plant development thereby artificially increasing the plant output molecules[9]. For the clonal propagation of plants with high medicinal value, the use of controlled environment systems is ideal due to the highly regulated growth conditions of water , light, temperature and soil. The use of greenhouses and *in vitro* techniques of growing plants.both presents the controlled environment systems. Propagation of plants under *in vitro* conditions is the use of a sterile, controlled environment in which plants are provided all the necessary nutrients through a pathogen free medium.[10] *In vitro* propagation has also been used in different medicinal plants . Being a unique tool, tissue culture provides more maintainance of numerous plant species or germplasm lines in a effective manner at very young growth stage which can be later on transferred into greenhouse environments[10] . Changes of media components and external inputs can increase production of medicinal constitutes[11]. Different

studies has been done on the micropropagation of Tulsi. Culturing of shoot tip culture is widely used for rapid propagation of many species due to its great advantages with respect of traditional methods . Young shoot cultured in Murashige and Skoog, (MS) medium containing sucrose and different growth regulators like as indole-3 acetic acid (IAA), α-naphtholene acetic acid (NAA), 6-benzyl aminopurine (BAP) forms callus which further elongated by transferring to fresh media after a fixed time interval. The medium containing BAP results into the maximum number of shoots. Rooting of shoot can be achieved by using MS medium adjuncted with 3% sucrose and 2.0 mg/l IAA Well developed plantlets can be transferred in polycup containing sterile soil having compost material and finally established in the field with 65-75% survival rate.

Establishment of Cultures

Establishing a protocol to regenerate, multiply and clone plants *in vitro* requires establishment of a sterile cultures of meristematic tissues such as shoot tips and nodal segments which can be obtained from seeds germinated *in vitro* or from field or greenhouse grown plants. The process of culture establishment is accomplished by surface sterilization to remove pathogens using alcohol, calcium hypochlorite, mercuric chloride and many others[10] Surface sterilization is followed by washing and transfer to growth medium containing essential nutrients such as water, sugar, micro and macro nutrients and plant growth regulators[10]. Many media compositions have been previously established based on basic plant requirements, but the optimized ratios and concentrations of those components are usually at least slightly variable based on tissue type, species and method of propagation[11]. Seeds of Tulsi can be successfully germinated on moistened filter paper on Petri plates[12,13] or on solidified media containing MS[14] salts, with a combination of BA (6-benzylaminopurine) (4.43 μM) and IAA (indole-3-acetic acid) (0.57 μM)[15]

Pre-sterilization washes were done in some of the studies and were variable in concentration and time. For example, mature shoot and leaf tips were washed with water and savlon (1%) for 20 minutes prior to sterilization[16] and axillary buds with a 5 minute rinse of laboline and sodium hypochlorite, followed by a water rinse before sterilizing.[17] Pattnaik and Chand also used axillary buds and only did a prewash with water prior to surface sterilization with a combination of sodium hypochlorite, sodium chloride, sodium hydroxide and sodium carbonate for 10 minutes before rinsing with sterile water[18]. A prewash with water and detergent can be done on inflorescences before surface sterilization and found to require a medium containing MS salts and 2, 4D (2,4-dichlorophenoxyacetic acid) at different concentrations for explant establishment in culture[19] Leaf and inflorescences explants shows the requirement of MS salt media with different plant growth

regulators to survive establishment after prewash with water before sterilization[20,21]. By contrast, Xiong employed a 30 minute pre-rinse followed by a 25% Clorox bleach solution for 10 minutes for culturing leaves on a basal medium[22]. None of these studies provided data for the contamination rates or survivability of explants after sterilization, indicating that contamination and death of tissue are minor issues in holy basil culture possibly due to its natural antimicrobial properties inhibiting infection. After establishment of sterile cultures, the manipulation of media to increase multiplication for clonal propagation can be investigated.

Shoot proliferation and multiplication

The process of shoot proliferation and multiplication requires the addition of various plant growth regulators. Plant multiplication can be through direct or indirect shoot proliferation, or stimulation of nodal explants. Nodal explants with axillary buds elongate and form shoots *in vitro*, which can be subcultured for further cloning of plants[10] Adventitious shoots can also be derived directly from explants or indirectly through callus cultures. Indirect reproduction is generally unfavourable for medicinal plant propagation, as the callus formation can lead to genetic instability and somaclonal variation. The shoot cultures are maintained in media generally supplemented with naturally occurring cytokinins such as kinetin (KN), zeatin, and iP (4-hydroxy-3-methyl-trans-2-butenylaminopurine) or synthetic cytokinins, e.g., BA and thidiazuron (TDZ) [23]Cytokinins generally counter the apical dominance from auxins, and encourage cell division, differentiation, increase adventitious shoot and leaf development, and decrease rooting [24.] For tissue culture, the concentration or source of the cytokinin required varies with plant species, age and growth stage. This is due to the variation in endogenous levels of hormones affecting the requirements to encourage multiplication[10] Cytokinins may also function in combinations with other growth regulators such as gibberellic acids or auxins depending on the individual plant species or genotypes. The use of nodal, direct and indirect methods has been explored using various explant materials in holy basil for shoot initiation.

Indirect Shoot Regeneration

Various explants of holy basil such as inflorescence, nodal, leaf, stem, shoot tip, axillary buds and cotyledons have been used to initiate callus cultures using BA or TDZ[19] or 2,4-D (1.0 mg/L) in combination with kinetin[21] Nodal segments were induced to form callus and shoots with the combination of auxin and cytokinins, NAA (naphthalene acetic acid) (5 mg/L) and BA (0.5 mg/L) or 2,4D at 0.2 mg/L[25] similar to multiplication from shoot tips with NAA 0.1 mg/L and BA at 0.2 mg/L[16] and axillary buds with NAA and kinetin[15] .Cotyledons have also been used as the explant

source for callus formation on medium with 2, 4 D and BA[12] Leaf explants are the most common source for callus initiation in holy basil using auxin-cytokinin combinations[20,21,22,16]. Callus formation in holy basil is quite varied in the response based on explant and plant growth regulators tested. However, the source material and time of harvest may play an important role in determining optimal levels of growth regulators for indirect callus formation. In some of these studies, the callus was further used to induce adventitious shoot regeneration. Callus from axillary buds required high levels of auxin (NAA at 12.42 µM) with low levels of cytokinin (2.32 µM of KN) to increase shoot buds, length and leaf number[15] . However, callus transferred onto cytokinin medium with 0.2 mg/L BA had 80% shoot regeneration[16] . Similarly, nodal explant callus formed multiple shoots with media containing BA (5 mg/L) with low levels of auxin (NAA 0.2 mg/L) in addition to glutamic acid at 50 mg/L[25] As well, cotyledon callus was used to initiate somatic embryogenesis with kinetin and IAA[12] .

Direct Shoot development from Explants

Direct shoot regeneration without an intervening callus from an explant is more desirable for propagating elite lines of a highly medicinal individual plant as the genetic stability is maintained. As with indirect regeneration, different explant sources have been used to initiate shoot response *in vitro*, including, peduncle, axillary buds, shoot tips, and nodal explants of holy basil. A higher ratio of cytokinin to auxin, or no additional auxin, in the medium is optimal for the majority of the explant material. For example direct shoot formation from inflorescence was achieved with BA (4.4 µM) in combination with IAA at low concentrations (0.05 mg/L)[19] whereas axillary buds showed optimum response with BA at 4.4 Mm[17]. Further enhancement in BA (4.4 µM) induced axillary bud proliferation was achieved with the addition of gibberellic acid at 0.5 mg/L[18]. In shoot tips and nodal explants, a combination of two cytokinins BA (4.4 µM) and KN (9.4 µM) was required to optimize shoot production[26].

Rooting and Hardening

Once established and multiplied, *in vitro* developed plants need to be hardened, or acclimatized before transfer to greenhouse conditions. Acclimatization is necessary to gradually reduce the influence of optimized environments provided for *in vitro* growth including high humidity, all the essential nutrients, no invasive species or pathogens, little to no competition, optimized light and ideal temperatures[10]. The establishment of roots on *in vitro* shoots is the first step in ensuring acclimation as it allows plants to seek nutrients and water in soil while maintaining structural stability[10]. Auxins are known to induce lateral and adventitious root development, tropism, bud

growth, apical dominance, elongation, embryonic development, vascular tissue growth and phyllotaxis[27]. Naturally occurring auxins including IAA and indole-3-butyric acid (IBA) as well as synthetic analogue such as NAA are commonly used to encourage root production *in vitro*. Concentrations and ideal auxin sources, whether natural or synthetic, are dependent on the plant species, and endogenous hormone levels. Optimum concentrations of auxin and cytokinins need to be determined to promote adventitious root growth, as the ratios of auxin to cytokinin can significantly influence root development[11]. For holy basil, both direct and indirect formation of roots has been observed with and without the addition of plant growth regulators[20,26,25,16]

Indirect Root Formation

Indirect root formation in holy basil rhizogenic callus was obtained from leaf explants cultured on medium containing 2 mg/L of NAA with 0.2 mg/L KN[20]. Transfer of this callus onto solid media containing 1.5 mg/L 2, 4D with 0.5 mg/L NAA, or the removal of cytokinins, resulted in optimized root development after three weeks. Afterwards, transfer of callus to liquid medium, containing high levels of NAA (1.3 mg/L) with the addition of BA (1.3 mg/L) increased the root production. This study demonstrated that both callus and roots can be generated using liquid culture.

Direct Root Formation

Direct root formation on micropropagated shoots of holy basil also requires a higher auxin to cytokinin ratio and is accomplished by the addition of NAA. Shoots derived from axillary buds were found to root optimally in media containing 1.0 mg/L NAA, with 98% root formation after 10-12 days[18,26] A similar concentration of NAA (1.5 mg/L) was used in shoot explants derived from callus tissue[25]. It was also observed that 90% root formation was achieved with considerably lower concentrations (0.1 and 0.2 mg/L) of NAA than those reported previously[16]. The use of auxins in combinations with cytokinins also produced significant root production in shoots. Concentrations of NAA at 26.85 µM and KN at 2.32 µM had improved root formation over other auxin (IAA) and cytokinins (BA)[15]. Interestingly, shoots from inflorescence explants were observed to root best in media containing no additional hormones[19]. With both direct and indirect methods for root formation in holy basil, high success rates were obtained. Overall, the use of high auxin to cytokinin ratios, specifically the use of the auxin NAA, improved root production which is a common observation in most plant species.

Acclimatization and Transfer to Greenhouse

In vitro raised plants often need acclimatization prior to transplant in greenhouse or field conditions as they are adjusted to *in vitro* conditions of high humidity and abundant resources.

Thus, plants may have poorly developed stomata on leaves and may not be completely autotrophic due to sugar supply in the media[10] . *In vitro* grown plants are generally removed from culture conditions, rinsed and transferred to soil in the greenhouses with high humidity through misting and low light intensity. After a slow acclimatization through reducing humidity and increasing the light intensity, plants become fully autotrophic and established to survive independently.

In holy basil, survival rates of *in vitro* grown plants are generally high and with a short acclimatization process. Transplant of longer shoots with roots into soil containing vermiculite and garden soil at a 1:1 ratio, showed 85% survival after 2 weeks[19]. In another acclimatization method by Pattnaik and Chand (1996), plants were transferred into growth chambers with high humidity for 2 weeks prior to greenhouse transfer with survival rates of 75 - 80% in the growth chamber, 80-85% after transfer to greenhouse followed by a 100% survival in natural conditions. Another study found a variable survival rating in different soil compost mixtures for holy basil, ranging from 12 to 82% with highest success rate after four weeks in soil and cow dung and the lowest survival in all other forms of compost[15]

Additional factors affecting Micropropagation

Medicinal plants respond normally to commonly used micropropagation treatments but some variations are possible. Browning of cultures is common due to high levels of phenolics in medicinal plants. By using polyphenol inhibitors, absorbents or through frequent subculturing the occurrence of browning can be alleviated[11] *In vitro* culture environment conditions are also a factor in the propagation process. A pH of 5.5 - 6.0 in the media allows for optimal absorption of nutrients which may change in autoclaving or due to the uptake and release of compounds by the plant[10]. Gelling agents such as gellan gum or agar to create a semisolid matrix for plants to remain upright in culture but can affect plant health[10]. Environmental conditions including the quality of light (35-112µmolm^{-}2s^{-}1) and day length (12-16h) with a temperature range between 22-25^{o}C are considered reasonable for tissue culture[11] .

A unique process for large-scale production of medicinal plants is the use of bioreactors, which provide a self-contained, sterile growth environment for large-scale propagation of shoots, roots and whole plants in a short time frame. Medicinal plants can be rapidly multiplied in various air-lift bubble-type bioreactors or temporary immersion bioreactors[28,29,30] These bioreactors use a liquid rather than solidified media method to promote biomass growth and the production of secondary metabolites[31]. Different designs of bioreactors are currently available including temporary immersion systems that allow for easy replacement of media, decrease cost of labour

and space, increase secondary metabolite production and multiplication of plants[31,32] . Bioreactors have been used in medicinal plants such as ginseng, garlic, St. John's Wort and sweet basil[31,33,34] but mass production in holy basil plants with liquid media has not been explored except for root development[20]

Pharmacological activity of *Ocimum sanctum*

Antimicrobial activity

Evaluation of antimicrobial activity of *Ocimum sanctum* leaf extract in normal tap water and local river water with different concentration (100 to 600 mg l^{-1}) reveals that 600 mg l^{-1} 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^{-1} of extract treated water showed 95-98% antibacterial activity in 14 to 16 hrs. The minimum bacterial concentration (MBC) was observed in 500 and 600 mg l^{-1} extract concentration. The concentration of the bacterial cells inhibited gradually for an hour was studied by spread plate method[8] .

The maximum antibacterial activity was observed at 600mg l^{-1} concentrated water treatment in both sample at the pH range of 6.8-7.0 for 15 to 16 hrs concluded that the human harmful organisms were inactivated by this plant extract at 600 mg l-1 in 15 to 16 hrs.

Toxicological studies

Acute and subacute toxicity studies with orally administered 50% ethanolic leaves extract of *Ocimum sanctum* Linn (OSE) to the four groups of mice with doses of 200, 600, and 2000 mg/kg, and general behavior, adverse effects, and mortality were recorded for up to 14 days. In subacute toxicity study, rats received OSE by gavage at the doses of 200, 400, and 800 mg/kg/day for 28 days, and biochemical, hematological, and histopathological changes in tissues (liver, kidney, spleen, heart, and testis/ovary) were determined. OSE did not produce any hazardous symptoms or death and CNS and ANS toxicities in the acute toxicity test. Subacute treatment with OSE did not show any change in body weight, food and water consumption, and hematological and biochemical profiles. In addition, no change was observed both in macroscopic and microscopic aspects of vital organs in rats. Study showed that *Ocimum sanctum* extract could be safe for human use[35]

Acute and subacute oral toxicities profile of *Ocimum sanctum* Linn. leaves reveals that it could be very useful in its future clinical study. The 50% ethanol extract of *Ocimum sanctum* leaves seemed to be nontoxic after its acute and subacute oral administrations. Further, teratogenic, mutagenic and carcinogenic studies with this plant are needed to complete the safety profile of this plant.

Hepatoprotective

In a study of the hepatoprotective potentials of *Ocimum sanctum* Linn. in lead induced toxicity. Aqueous extract of *O. sanctum* was prepared as per protocol. Wistar strains of Albino rats were used as the experimental models. Animals were grouped into six comprising of six rats each. Hepatotoxicity was induced using lead. The selected plant drug was administered to the animals orally for a period of 21 days. The Hepatic serum markers AST, ALT, ALP, GGT, Serum Protein, Serum Bilirubin and Tissue Glycogen were analyzed. The antioxidant status of the animals was also assessed in the animals by measuring the activity of GSH and SOD. The extent of lipid per oxidation (LPO) was also measured. The induction of liver injury with lead resulted in significant raise in the serum marker enzyme level along with an increase in the serum bilirubin content. The inactivation of the liver was evident from the lowered levels of serum protein and tissue glycogen levels. The antioxidant status was very low thus causing an accumulation in lipid peroxides in the hepatic tissues. All the parameters studied were restored to near normal when treated with the aqueous extract of *O.sanctum* depictiing the hepatoprotective nature of *O.sanctum* in lead induced toxicity. The protective activity may be attributed to the antioxidant activity of the plant[36].

Antibiotic property

Essential oils extracted from the leaves of *Ocimum sanctum L.* has been found to inhibit growth of *E. coli, B. anthracis* and *P. aeruginosa* in-vitro, showing its antibacterial activity. *Ocimum sanctum* also possesses antifungal activity against *Asperigillus niger* and aqueous extract of it was found to be effective in patients suffering from viral encephalitis[37] In the treatment of ring worm infections, Tulsi leaves paste is indeed found to be very effective. Tulsi has significant natural antibacterial, antiviral and antifungal activities and is helpful in treating many serious systemic diseases, as well as localized infections[38]

Hypoglycemic, Hypolipidemic and Antioxidant properties

The aqueous extract of *Ocimum sanctum* mixed with diet for eight weeks to diabetic (streptozotocin induced) rats were studied. There was significant reduction in fasting blood glucose, serum lipid profile, lipid peroxidation products, and improvement in glucose tolerance. The aqueous extract also decreased LPO formation (thiobarbituric acid reactive substances TBARS) and increased antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione transferases (GT). It also increased antioxidant like reduced glutathione (GSH) levels in plasma and liver, lung, kidney and brain of rat. Tulsi has been found to have therapeutic potential as antidiabetic, hypolipidemic, and antioxidant medicine[39].

Hepatoprotective, Renoprotective and Neuroprotective activities

Ocimum sanctum leaf extract was found to be hepatoprotective against hepatotoxic paracetamol by significant reduction of serum enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) in rats and also showed marked reduction in fatty degeneration of liver on histopathological examination[40]. Administration of combination of *Ocimum sanctum* aqueous leaf extract and gentamicin, significantly prevented rise in levels of serum creatinine and blood urea when compared to the gentamicin only treated group in rats[41] Leaves and seeds of Tulsi plant have been reported to reduce blood and urinary uric acid level in albino rabbits and also possessed diuretic property[42]. *Ocimum sanctum* leaf extract prevents stress induced dendritic deficiency in hippocampal neurons in albino rats[43]. Research indicate that Tulsi has a very high safety margin and very low toxic profile, providing safe beneficial effects at low doses without any undesirable side effects. In Ayurvedic medicine, Tulsi has therapeutic potential either alone or in combination with other plants in various clinical conditions like eye disorders (glaucoma, cataract, & chronic conjunctivitis), catalepsy, snake and scorpion bites etc[38,37].

Antioxidant and wound healing effects

In a study to assess the potential of alcoholic and aqueous extracts in wound healing in Wistar albino rats, the rats were divided into five groups of six animals each. Group 1 is normal wounded control and the other four groups were treated with two different doses each of alcoholic and aqueous extract of O. sanctum. The wound healing parameters were evaluated by using incision, excision and dead space wounds in extract-treated rats and controls. Both the doses of alcoholic and aqueous extract significantly increased wound breaking strength, hydroxyproline, hexuronic acid, hexosamines, superoxide dismutase, catalase, reduced glutathione and significantly decreased percentage of wound contraction and lipid peroxidation when compared with the control group. The results shows that *Ocimum sanctum* has antioxidant properties, which may be responsible and favorable for faster wound healing and this plant extract may be useful in the management of abnormal healing and hypertropic scars[44].(

Anti-oxidant bioassay-directed extraction of the fresh leaves and stems of *Ocimum sanctum* and purification of the extract yielded the following compounds; cirsilineol[35], cirsimaritin[45] , isothymusin[36] , isothymonin[38], apigenin[39], rosmarinic acid[38], and appreciable quantities of eugenol. The structures of compounds 1-6 were established using spectroscopic methods. Compounds 1 and 5 were isolated previously from *O. sanctum* whereas compounds 2 and 3 are here identified for the first time from *O. sanctum*. Eugenol, a major component of the volatile oil, and compounds 1, 3, 4,

and 6 demonstrated good antioxidant activity at 10-μM concentrations. Anti-inflammatory activity or cyclooxygenase inhibitory activity of these compounds were observed. Eugenol demonstrated 97% cyclooxygenase-1 inhibitory activity when assayed at 1000-μM concentrations. Compounds 1, 2, and 4–6 displayed 37, 50, 37, 65, and 58% cyclooxygenase-1 inhibitory activity, respectively, when assayed at 1000-μM concentrations. Eugenol and compounds 1, 2, 5, and 6 demonstrated cyclooxygenase-2 inhibitory activity at slightly higher levels when assayed at 1000-μM concentrations. The activities of compounds 1–6 were comparable to ibuprofen, naproxen, and aspirin at 10-, 10-, and 1000-μM concentrations, respectively[46]

Anticancer property of *Ocimum sanctum*

The fresh leaf of the *Ocimum sanctum* has been shown to enhance the immunity and also to possess anti carcinogenic properties in experimental animals[47]. Methanolic extract of Ocimum varieties have been shown to possess cancer preventive activities through reduction of excess amount of nitric oxide.[48]Tulsi has been found to decrease the incidence of benzo (a) pyrine-induced neoplasia and 3-methyl di-methyl amino azobenzene, induced hematomas in experimental animals.[49] Furthermore, the anticancer activity of OS has been reported against human fibrosarcoma cells culture. Morphologically, the cells showed shrunken cytoplasm and condensed nuclei and the DNA was found to be fragmented on observation in agarose gel electrophoresis[50]. Several studies have shown that *Ocimum sanctum* possess prominent anticancer activity[51,52]

Adaptogenic/ antistress properties

Ocimum sanctum has been found to be a powerful adaptogenic / antistress agent, helpful in preventing and reducing stress: mental, emotional, physical, and environmental stress[53,54]. [. The immunostimulant capacity of *O. sanctum* may be responsible for the adaptogenic action of plant[6]. The experimental studies on animal models have shown that *O. sanctum* leaves produced significant increase in the levels of enzymatic (superoxide dismutase) and nonenzymatic (reduced glutathione) antioxidants which have the therapeutic potential in prevention and treatment of Cancer. Antistressor activity of *O. sanctum* is partly attributable to its antioxidant properties[55]

Immunomodulatory Activity

Ocimum sanctum has potential to modulate the humoral immune responses by acting at various levels in the immune mechanisms such as antibody production, release of mediators of hypersensitivity reactions, and tissue responses to these mediators on the target organs[56,57,58]. have found immunotherapeutic potential of aqueous extract of *O. sanctum* L. leaf in bovine sub-clinical mastitis (SCM) which was investigated after intra-mammary infusion of aqueous extract and the

results revealed that the aqueous extract of *O. sanctum* treatment reduced the total bacterial count and increased neutrophil and lymphocyte counts with enhanced phagocytic activity and phagocytic index. In another study Mediratta PK have investigated the immunomodulatory effect of *O. sanctum* L. seed oil (OSSO) on some immunological parameters in both non-stressed and stressed animals and evaluated that OSSO appears to modulate both humoral and cell-mediated immune responsiveness and these immunomodulatory effects may be mediated by GABAnergic pathway[59]

CONCLUSION

Tulsi is one of the most sacred herbs of India, and is an integral part of ancient Hindu traditions. According to Hindu mythology, Tulsi has been originated as one of the 14 "Ratnas (gems or treasures)" from the ocean as the ultimate sacred plant to enhance health and remove diseases. It is evident from the above review that significant progress has been made on various medicinal and aromatic plant species including *Ocimum sanctum* .Research in these areas frequently lead to vast improvement in yield of important plant derived compounds. Tissue culture methodology can be particularly useful in achieving rapid multiplication of the higher yielding individual plants. Micro propagation assist with production of plants, but also allows for the maintenance of large populations that can be further analyzed for their medicinal value.

Demand for natural products for fragrances, dyes and several pharmaceutical compounds are increasing day by day and technology should be ready to meet the ever increasing demand in coming decades. This will help to bridge the gap between ever increasing demand and supply of raw products necessary for pharmaceuticals, medicinal, food, cosmetic industry.

REFERENCES

1. Latha S, Kakkar Srivastava VK Saxena KK Saxena KS and Kumar A. "A comparative antipyretic activity of Ocimum sanctum, Glycerrhiza glabra and aspirin in experimentally induced Pyrexia in rats" .Indian J.Pharmacol. 1999; 33: 71-74.
2. Uma Devi P and Gunasoundari A. "Radioprotective effects of leaf extract of Indian Medicinal plant Ocimum sanctum." Indian J.Exp.Biol.1995; 33: 205.
3. Somasekar Shetty B, Udupa AL and K.Bhaskar U. "Effect of Ocimum sanctum on Wound healing." Indian.J.Pharmacol. 1999; 31: 78-80.
4. Lawrence BM, Hogg JW, Terhune SJ, Pichitakul N. Essencial oils and their constituents. The oils of *O. sanctum* and *O. basilicum* from Thailand. Flav Industry 1972; 9:47-49.
5. Skaltsa H, Philians S, Singh M. Phytochemical study of the leavesof *Ocimum sanctum*. Fitoterapia 1987; 8: 286.

6. Godhwani S, Godhwani J L, Vyas DS. *Ocimum sanctum*-A preliminary study evaluating its immunoregulatory profile in albino rats. Journal of Ethnopharmacology 1988; 24: 193-198.
7. Mukherjee R, Dash PK, Ram GC. Immunotherapeutic potential of *Ocimum sanctum* (L) in bovine subclinical mastitis. Res Vet Sci. 2005; 79:37-43.
8. Atal CK and Kapoor BM. Cultivation and utilization of medicinal plants (Eds.PID CSIR), 1989.
9. Dhar M L, Dhar M M, Dhawan BN, Mehrotra BN & Roy C.Screening of Indian plants for biological activity, Part I. Indian Journal of Experimental Biology 1968,6 232–247.
10. George EF, Hall MA and De Klerk, GJ. *Plant Propagation by Tissue Culture: Volume 1. The Background*. 3rd ed. Springer Netherlands.2008.
11. Rout GR, Samantaray S and Das P. *In vitro* manipulation and propagation of medicinal plants. *Biotechnology advances.*1999; 18: 91–120.
12. Mathew R. Comparison of somatic embryo formation in *Ocimum basilicum L.*, *Ocimum sanctum L*. and *Ocimum gratissimum*. International Journal of Pharma and Bio Sciences.2011; 2: 356–367
13. Tyub S and Kamili A. Enhanced Axillary shoot proliferation in *Ocimum sanctum* Linn. via shoot tip culture using various concentrations of BAP. J. Res. Dev.2009; 8: 80–85.
14. Murashige T and Skoog F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Physiologia Plantarum. 1962; 15: 473–497
15. Gogoi K and Kumaria S. Callus - mediated plantlet regeneration of Ocimum tenuiflorum L . using axillary buds as explants. Plant Science. 2011; 2: 001–005.
16. Banu L and Bari M. Protocol establishment for multiplication and regeneration of *Ocimum sanctum Linn.*: an important medicinal plant with high religious value in Bangladesh, J. Plant Sci.2007; 2: 530–537
17. Mandal J, Pattnaik S and Chand P K. Alginate encapsulation of axillary buds of *Ocimum americanum* L. (hoary basil), *O. Basilicum* L. (sweet basil), *O. Gratissimum* L. (shrubby basil), and *O. Sanctum.* L. (sacred basil). In Vitro Cellular and Developmental Biology - Plant 2000; 36: 287–292.
18. Pattnaik S. and Chand PK. In vitro propagation of the medicinal herbs *Ocimum americanum L*. syn. *O. canum Sims.*(hoary basil) and *Ocimum sanctum L*.(holy basil). *Plant Cell Reports.*1997; 15: 846–850.

19. Singh NK and Sehgal CB. Plant Growth Regulation November 1999, Volume 29, Issue 3, pp 161-16 Micropropagation of 'Holy Basil' (Ocimum sanctum Linn.) from young inflorescences of mature plants
20. Shilpa K, Selvakkumar C, Senthil AK and Lakshmi BS. *In vitro* root culture of *Ocimum sanctum L.* and evaluation of its free radical scavenging activity. Plant Cell, Tissue and Organ Culture (PCTOC).2009; 101: 105–109.
21. Hakkim FL, Shankar CG and Girija S. Chemical composition and antioxidant property of holy basil (*Ocimum sanctum L.*) leaves, stems, and inflorescence and their in vitro callus cultures. Journal of agricultural and food chemistry. 2007; 55: 9109–9117.
22. Xiong Lim Z, Pick Kiong Ling A and Hussein S. Callus induction of *Ocimum sanctum* and estimation of its total flavonoids content. Asian Journal of Agricultural Sciences. 2009; **1:** 55–61.
23. Jones MP, Murch S J and Saxena PK. Thidiazuron-induced regeneration of *Echinacea purpurea L.*: micropropagation in solid and liquid culture systems. Plant cell reports. 2007; 26: 13–19.
24. Taiz L and Zeiger E. (2010). *Plant Physiology*. Sinauer Associates, Incorporated
25. Shahzad A and Siddiqui SA In vitro organogenesis in *Ocimum sanctum*, a multipurpose herb. Phytomorphology. 2000; 50: 27.
26. Girija S, Kavitha S and Deepavathi S. Direct multiple shoot regeneration from shoot tip and nodal explants of *Ocimum sanctum L.*(Tulsi): A medicinal herb. Plant Cell Biotechnol. Mol. Biol. 2006; 7: 23–28.
27. Woodward AW and Bartel B. Auxin: regulation, action, and interaction. Annals of botany. 2005; 95: 707–35.
28. Murch SJ, Ragone D, Shi WL, Alan AR and Saxena PK. In vitro conservation and sustained production of breadfruit (*Artocarpus altilis*, Moraceae): modern technologies for a traditional tropical crop. Die Naturwissenschaften 2008; 95:
29. Jones AM, Saxena PK and Murch SJ. Elicitation of secondary metabolism in *Echinacea purpurea L.* by gibberellic acid and triazoles. Engineering in Life Sciences. 2009; 9: 205–210.
30. Abbasi BH, Liu R, Saxena PK and Liu CZ. Cichoric acid production from hairy root cultures of *Echinacea purpurea* grown in a modified airlift bioreactor. Journal of Chemical Technology and Biotechnology. 2009; 84: 1697–1701.

31. Paek KY, Chakrabarty D and Hahn EJ. Application of bioreactor systems for large scale production of horticultural and medicinal plants. Plant Cell, Tissue and Organ Culture. 2005; 81: 287–300.
32. Thatoi H and Patra JK. Biotechnology and Pharmacological Evaluation of Medicinal Plants: An Overview. Journal of Herbs, Spices and Medicinal Plants. 2011; 17: 214–248.
33. Zobayed S and Saxena PK. Production of St. John's wort plants under controlled environment for maximizing biomass and secondary metabolites. In Vitro Cellular and Developmental Biology – Plant. 2004; 40: 108–114.
34. Kintzios S, Kollias H, Straitouris E and Makri O. Scale-up micropropagation of sweet basil (*Ocimum basilicum* L.) in an airlift bioreactor and accumulation of rosmarinic acid. Biotechnology letters. 2004; 26: 521–523.
35. Gautam MK and Goel RK. 2014. Toxicological Study of Ocimum sanctum Linn Leaves: Hematological, Biochemical, and Histopathological Studies. Journal of Toxicology. Volume 2014.
36. Akilavalli N, Radhika J and Brindha P. 2011. Hepatoprotective activity of *ocimum sanctum* linn. against lead induced toxicity in albino rats Asian Journal of Pharmaceutical and Clinical Research4; 2.
37. Sen P, Maiti PC, Puri S, Ray A, Audulov NA and Valdman AV. Mechanism of anti-stress activity of *Ocimum sanctum Linn Eugenol* and *Tinospora malabarica* in experimental animals. Indian J Exp Biol. 1992; 30 (7): 592-596.
38. *Ocimum sanctum.* The Indian home remedy. In: Current Medical Scene, March-April 1992 (Edited and published by S. Rajeshwari, Cipla Ltd., Bombay Central, Bombay).
39. Hussain EHMA, Jamil K and Rao M. Hypoglycemic, hypolipidemic and antioxidant properties of Tulsi (*Ocimum sanctum*) on streptozotocin induced diabetes in rats. Indian J of Clin Biochemistry, 2001; 16(2): 190-194.
40. Chattopadhay RR, Sarkar SK, Ganguly S, Medda C and Bassu TK. Hepatoprotectivity activity of *Ocimum sanctum* leaf extract against paracetamol induced hepatic damage in rats. Indian J Pharmacol. 1992; 24: 163-165.
41. Muglikar AG, Kothekar MA, Chilwant KS and Jaju JB. Effect of *Ocimum sanctum* (OS) aqueous leaf extract on gentamicin induced nephrotoxicity in rats. Indian J Pharmacol. 2004; 36(2): 123.
42. Sarkar A, Pandey DN, Pant MC. A report on the effect of *Ocimum sanctum* (Tulsi) leaves

and seeds on blood and urinary uric acid, urea and urine volume in normal albino rabbits. Indian J Physiol Pharmacology. 1990; 34: 61–62.

43. Rodrigues V, Rao MS, Satish N, and Rao GM. *Ocimum sanctum* prevents stress induced damage to the hippocampal CA-3 neurons in the rats. J Anat Soc India. 2001; 50(1): 70.
44. Somashekar S, Saraswati U and Laxminarayana U.Evaluation of Antioxidant and Wound Healing Effects of Alcoholic and Aqueous Extract of Ocimum sanctum Linn in Rats Advance Access Publication 4 April 2007.
45. Atal CK. Queen of herbs tulsi (*ocimum sanctum*) removes impurities from water and plays disinfectant role Babita Labh Kayastha. Journal of Medicinal Plants Studies. 2014, Volume: 2, Issue: 2
46. Kelm MA, Nair MG and Strasburg GM. Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum* Linn., D.L. DeWitt.
47. Mondal S, Mirdha BR, Mahapatra SC. The Science behind sacredness of Tulsi (*Ocimum sanctum* Linn.). Indian J Physiol Pharmacol. 2009; 53(4): 291-306.
48. Kim OK, Murakami A, Nakamura Y, Ohigashi H. Screening of edible Japanese plants for nitric oxide generation inhibitory activities in RAW 246.7 cells. Cancer let. 1998; 125(1-2):199-207.
49. Aruna K, Sivaramkrishnan VM. Plant products as protective agents against cancer. Ind. J. Exp. Biol. 1990; 28(11):1008-1011
50. Kathiresan K, Guanasekan P, Rammurthy N, Govidswami S. Anticancer activity of *Ocimum sanctum.* Pharmaceutical Biology1999; 37(4):285-290.
51. Govind P. An overview on certain anticancer natural products. J Pharm Res. 2009; 2(12): 1799-1803
52. Govind P, Madhuri S. Autochthonous herbal products in the treatment of cancer. Phytomedica 2006; 7:99-104.
53. Singh N, Gupta ML, Das M, Kohli RP. Preventive effect of some Indigenous drugs on stress and aspirin induced gastric ulcer in albino rats. In the Proceeding Decennial Conference. Ind. Pharmacol. Sos, Calcutta 1977, Abstract No. 171.
54. Singh SP, Singh N. Experimental Evaluation of Adaptogenic Properties of *Ocimum sanctum*. Ind J Pharmacol. 1978; 10:74.
55. Jyoti S, Satendra S, Sushma S, Anjana T, Shashi S. Antistressor activity of *Ocimum sanctum* (Tulsi) against experimentally induced oxidative stress in rabbits. Methods Find

Exp Clin. Pharmacol. 2007; 29(6):411-6.

56. Mediratta PK, Dewan V, Bhattacharya SK, Gupta VS, Maiti PC, Sen P. Effect of *Ocimum sanctum* Linn on humoral immune responses. Indian J Med Res. 1988; 87:384-6.
57. Satayavanti GV, Raina MK, Sharma M. Medical plants of India published by ICMR, New Delhi, 1976.
58. Mukherjee R, Dash PK, Ram GC. Immunotherapeutic potential of *Ocimum sanctum* (L) in bovine subclinical mastitis. Res Vet Sci. 2005; 79:37-43.
59. Medrita PK, Sharma KK. Effect of essential oil of the leaves and fixed oil of the seeds of *Ocimum sanctum* on immune responses. J Med Aro Plant Sci. 2000; 22:694-700.