Tulsi (*Ocimum sanctum*) – a myriad medicinal plant, secrets behind the innumerable benefits

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Abstract: Tulsi (*Ocimum sanctum*) is an omnipresent, multipurpose plant and regarded as holy plant in Hindu religion finds place in front of every Hindu household. This herb is a member of Lamiaceae family, well known for its spiritual, religious and medicinal importance in India. Scientific investigations of traditional belief of medicinal properties of Tulsi have got impetus mostly in the later part of the 20th century. Characteristically, the plant imparts a pungent, bitter, hot, light and dry effect indicating the presence of curative value. It is extensively famous and has been used since centuries in Ayurvedic and Greek medicines owing to its beneficially diverse properties. The scientific fact behind these benefits owe to the presence of phytochemicals in leaves like flavonoids, phenol, terpenoids, alkaloids, eugenol and others which impart ability to overcome the infection by the target cells. The ability can be antimicrobial, pharmacological, any therapeutic, nutritional value or even leveraging of certain levels of phytochemicals thus bringing about the cellular protection. This review is an attempt to present brief information available on the religious values, studies on physiological aspects, antimicrobial properties, pharmacological application, therapeutic uses, phytochemical analysis, animal nutrition supplement and biotechnological studies comprising molecular marker usage, genome sequencing, bioinformatics and nanotechnological studies in Tulsi.

Keywords: Tulsi, *Ocimum sanctum*, antimicrobial, therapeutic, molecular studies

Introduction

Religious values of Tulsi

Tulsi, *Ocimum basilicum* or the holy basil holds a prominent place in Indian Hindu culture and traditions. It is used on almost all religious occasions and is often presented to the gods in the form of garlands (Desi wisdom online, 2018). Tulsi is one of the oldest aromatic herbs of the family Lamiacaceae. It is popularly known as “holy basil or sacred basil” and is present in almost every household in Indian sub-continent due to its medicinal, nutritional and spiritual properties. It is also

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known as “the incomparable one, mother of natural medicine and the queen of herbs” (Ashok *et al*, 2018). Several species of basil, especially "tulsi" (*Ocimum sanctum* L.), are regarded as the most sacred plants in the Hindu religion and therefore Basil is found in almost every Hindu house throughout India. The leaves are used for different ceremonies such as births, weddings, sacred rituals, as well as in funerals (Majupuria and Joshi, 1997; Pandey, 1989; Simoons, 1998).

Physiological studies in Tulsi

The ecological dominance of Sri Tulsi over Krishna Tulsi is indicated by the differences observed in photosynthetic efficiency by Batra and his co-workers (2016). The study was on comparison of the overall adaptiveness of two *Ocimum sanctum* (holy basil) morphotypes, *viz*., Sri Tulsi and Krishna Tulsi. In this synecological assessments, comparative ecophysiological studies are been done by measurement of photosystem II (PSII) chlorophyll a fluorescence, pigment composition, and proline content. Results state that Sri Tulsi possess 30 % higher effective quantum yield of PSII (ΔF/Fm’stat) and maximum apparent electron transport rate (ETRmax) than Krishna Tulsi. The authors also reported the presence of two fold higher chlorophyll and carotenoid content in Sri Tulsi than Krishna Tulsi.

Another study conducted from May, 2017 to October, 2017 for the choice of season for collection and extraction of two photosynthetic pigments, (Total Chlorophyll and total Carotenoids content) for pharmaceutical product preparation from *Ocimum sanctum* (Tulsi) (Subham *et al*, 2018). The study from different seasons indicates that the autumn season (October) would be the best with highest photosynthetic pigments contents and lower levels during rainy season (July). Thus, providing the suitable information for the utilization of this very useful medicinal plant fresh leaves for pharmaceutical and food industry to extract these pigments for herbal product preparations. These two photosynthetic pigments act as antioxidant and have the ability to fight against several types of cancer (Subham *et al*, 2018) and are also prescribed in case of skin and eye problems (Subham *et al*, 2018).

Antimicrobial studies in Tulsi

Antimicrobial activity pertains to the capacity of a substance to diminish or inhibit the growth of microorganisms. Different substances have different degree of antimicrobial activity. Several

medicinal properties have been attributed to the Tulsi plant not only in Ayurveda and Siddha but also in Greek, Roman and Unani systems of medicine (Babita, 2014). In Ayurveda, Tulsi used as antiasthmatic and antikaphic drugs (Babita, 2014). It is also used in treatment of fever, bronchitis, arthritis, convulsions etc. (Babita, 2014). The reports provided can provide more evidence for using tulsi leaves as water purifier. A study focused on 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}) of Tulsi leaf extract in tap and river water (Babita, 2014). 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 to 98% antibacterial activity in 14 to 16 hrs. The minimum bacterial concentration (MBC) was observed in 500 and 600 mg l^{-1}extract. The concentration of the bacterial cells inhibited gradually for an hour was studied by spread plate method.

Another study focused on to compare the antimicrobial activity of methanolic leaf extracts of tulsi and neem (Vipul *et al,* 2018). Here the leaf extracts of Tulsi (*Ocimum sanctum*) and neem (*Azadirachta indica*) were used to compare their antimicrobial activity toward Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Phytochemical extracts have been made by the use of methanol as solvent and dried leaf powder by the cold maceration extraction process. The phytochemical extracts of neem and tulsi were allowed to diffuse into the medium, and after incubation of 24 h at 37°C, the zones of inhibition were observed. Statistical analysis showed that tulsi was more effective toward *S. aureus* while neem was more effective toward *E. coli.* Minimum inhibitory concentration (MIC) of tulsi for both the microorganisms was 0.4 g/ml and the MIC of neem for both the microorganisms was 0.2 g/ml. When both the tulsi and neem extract were mixed with each other for every concentration at equal volume, they have revealed better effects in comparison to individual neem or tulsi extract and also the MIC got reduced to 0.2 g/ml for both the bacteria. Leaf extracts of both the *A. indica* and *O. sanctum* have shown antimicrobial activity against *E. coli* and *S. aureus. A. indica* has higher antimicrobial activity against *S. aureus*, whereas the *O. sanctum* was found to be more effective against *E. coli* (indicated by the zone of inhibition).

In the view of endemic avian influenza H9N2 infection in poultry, its zoonotic potential and emergence of antiviral resistance, two herbal plants, (*Ocimum sanctum* and *Acacia arabica*) which are easily available throughout various geographical locations in India were taken up to study their

antiviral activity against H9 N2 virus (Ghoke *et al,* 2018). Evaluation of antiviral efficacy of three different extracts (crude extract, terpenoid and polyphenol) each from leaves of *O. sanctum* and *A. arabica* against H9N2 virus was done using in ovo model. Three experimental protocols *viz.* virucidal (dose-dependent), therapeutic (time-dependent) and prophylactic (dose-dependent) activity in specific pathogen free embryonated chicken eggs and the reduction in antiviral efficacy determined with Haemagglutination (HA) and real time quantitative reverse transcription polymerase chain reaction (RT-qPCR) assays. Significant virucidal activity was found in all the extracts (crude extract, terpenoid and polyphenol) of *O. sanctum* and *A. arabica*, however, crude extractocimum and terpenoidocimum showed highly significant to significant ($p < 0.001$–0.01) decrease in virus genome copy numbers with lowest dose tested. Similarly, therapeutic effect was observed in all three extracts of *O. sanctum* in comparison to the virus control, nevertheless, crude extractocimum and terpenoidocimum maintained this effect for longer period of time (up to 72 h post-incubation). None of the leaves extracts of *A. arabica* had therapeutic effect at 24 and 48 h post-incubation, except the crude extractacacia and polyphenolacacia showed delayed therapeutic effect (72 h post-inoculation). Prophylactic potential is reported in polyphenolacacia with highly significant antiviral activity compared to virus control ($p < 0.001$).The crude extract and terpenoid isolated from the leaves of *O. sanctum* and polyphenol from *A.arabica* has shown promising antiviral properties against H9N2 virus. However the necessity of future investigations was stressed to formulate combinations of these compounds for achieving the broader antiviral activity against H9N2 viruses and evaluate them in chickens.

Staphylococcus aureus is among the pathogens capable of developing a broad spectrum of infections in human beings. Besides hospital, the bacterium is present in the community and has a high resistance to antibiotics, which is also increasing on an ongoing basis. Resistance to β-lactam antibiotic family is one of the concerns about the bacterium that has encountered the treatment of such infections with difficulty. Due to the increased resistance and importance of this bacterium, new strategies are needed to control this pathogen and much research is planned in this regard. Several studies have been carried out and are being designed using various herbs to find active ingredients to deal with this bacterium. The study of the antibacterial activity of different medicinal plants and the effects of their active ingredients on methicillin-resistant and methicillin-sensitive *S. aureus* (MRSA, MSSA) has been done to clarify the pathway to further studies in this regard (Askarinia *et al,* 2019).

The antibacterial activity of methanol extract of herbal plants against the multidrug resistant (MDR) Gram negative bacteria isolated from clinical samples (Bishnu *et al,* 2018). Gram negative bacteria isolated from various clinical samples were processed for antibiotic susceptibility test by modified Kirby-Bauer disc diffusion method and MDR bacteria were selected. Methanol extracts of six different medicinal plants *Acorus calamus* (bojho), *Ocimum sanctum* (tulsi), *Azadirachta indica* (neem), *Cinnamomum tamala* (tejpatta), *Aloe vera* and *Zanthoxylum alatum* (timur), were tested for antibacterial activity against the selected MDR bacteria by agar well diffusion method. From clinical samples, 8 different MDR Gram negative bacteria isolated were *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Citrobacter spp., Proteus mirabilis, Proteus vulgaris, Acinetobacter spp.* and *Pseudomonas spp.* with *E. coli* dominated the number. Out of six medicinal plants extracts, *Z. alatum, C. tamala* and *Ocimum sanctum* were found to be effective with zones of inhibition ranging from 9-13 mm. The medicinal plants with antibacterial activity can be an alternative source of medicine against MDR Gram negative bacteria. Several herbal plants extracts exhibit antibacterial activity against MDR Gram negative bacteria. Antibacterial activity of plant extracts can vary with type of plant and extraction methods. Thus, it proves that for optimal benefit of plant extract, an appropriate extraction method and use of purified product is essential.

Pharmacological application studies of Tulsi

A summarization work of botanical, pharmacological, phytochemical, ethno medicinal and toxicological information for helping researchers and clinicians of remedial effects is done by Bano and his colleagues (2017). The active biological compound mediating the therapeutic properties is mentioned as Eugenol (1-hydroxy-2-methoxy-4-allylbenzene). The data collected from various search sources like journals, Online published articles, internet sites, Scopus, Pubmed and Google Scholar indicates the uses of several parts of tulsi in Ayurveda and its recommendation in curing wide range of ailments listing dysentery, diarrhea, bronchitis, malaria, dermatological issues, eye ailments, rheumatoid arthritis and many more. Scientific proof for presence of several medicinal properties (anticancer, anti-diabetic, anti-fertility, antifungal, antimicrobial, cardio protective, analgesic, antiallergic, immunomodulatory, antioxidant, antispasmodic and adaptogenic, anti-inflammatory, antipyretic, antiviral, antiulcer, hepatoprotective, anti-arthritis and CNS depressant activities) are been discussed in detail.

Ocimum gratissimum, common name tulsi, is considered as a sacred plant and worshiped in India. It is a valuable medicinal plant which has numbers of pharmacological properties. Antitumor and anti-cancer effects have been reported in *in vitro* experiments (Sheelu *et al,* 2017). It is also recommended for treatment of diseases like bronchitis, bronchial asthma, diarrhea, dysentery chronic fever etc. Tulsi contains eugenol (1-hydroxy-2-methoxy-4-allylbenzene) which can be used for therapeutic purposes. Preclinical studies has revealed that certain compounds present in Tulsi like rosmarinic acid, apigenin, myretenal, luteolin, ß-sitosterol and carnosic acid, have antioxidant properties. Contents of secondary metabolites can be increased with the help of different tissue culture and transformation techniques. Availability of data on genome and transcriptome sequences can reveal the sites of gene for important secondary metabolite pathways.

Therapeutic uses of tulsi

A detailed review is presented by Pattanayak and team (2010) from the reported pharmacological studies to endorse the therapeutic values of *O. sanctum* L. The results presented in the review study will support best use of this plant for effective human and animal disease therapy, an importance source of bioactive substances and additionally supporting for ethno-botanical study. Traditional medical practitioners have widely used the medicinal plants in day to day practice for curing various diseases using different parts (leaves, stem, flower, root, seeds and even whole plant) of *Ocimum* sanctum Linn. These parts use has been recommended for curing of dysentery, diarrhea, bronchitis, arthritis, insect bites, malaria, skin disease, eye diseases and so on. Also reported on possession of antidiabetic, cardioprotective, antimicrobial, antispasmodic, anti-fertility, antifungal, anticancer, adaptogenic and analgesic actions. The main constituent Eugenol (1-hydroxy-2-methoxy-4-allylbenzene) was largely influencing the therapeutic value of *O. sanctum* L. Thus, leveraging the levels of eugenol or increase in raw material through genetic, molecular and *in vitro* culture tools may help to utilize this herb more efficiently in pharmaceutical industry.

Most of the traditional medicines used are originated from organic matter, minerals and medicinal plants. 2500 species of plants from 21,000 plants listed by World Health Organization (WHO) for medicinal purpose are in India, of which 150 species are largely used in commercial scale. This information makes India with largest medicinal herbs, thus is referred to as botanical garden of the world. Maurya and Srivastava (2011) have reviewed these aspects focusing on the plants and herbal drug preparations utilization for treatment of different chronic diseases across the world. They have indicated the rising numbers in the use of Ayurvedic medicines in day to day life among adults and

children in different parts of the world. The traditional uses of various medicinal plants like Tulsi, Arjuna, Ashwagandha and Neem and also the latest discovery and improvement in medicinal and aromatic plants for augmentation of health care of mankind are reported. Discussions on the use of herbal medicines with Antiulcer, Antipyretic, Anti-cancerous and Antidiabetic activity due to the presence of chief sources of therapeutic agents for curing human diseases from the various chemical compounds in medicinal plants are adding additional information.

Ocimum tenuiflorum is very well known as Krishna Tulsi, most sacred herb in India posses various healing medicinal properties for human life. This has evidenced with use of many parts of this plants such as stems, leaves, flowers in treatment of several disorders namely cough, cold, fever, swelling, vomiting, skin diseases etc. An updated review with scientific proof of medicinal properties against several disorders is highlighted by Palal Ravi and coworkers (2012). The specific chemical constituents listed in *Ocimum tenuiflorum* are methyl cinnamate, eugenol, thymol and camphor. Similarly the review has described on antimicrobial, anti-cancer, antifungal, antispasmodic, antiviral, analgesic, immuno-stimulatory and anti-inflammatory properties of tulsi.

Irrespective of the serotypes existing dengue fever is the most prevailing arthropod-borne viral diseases of the world population. Dengue vaccine development has difficulties due to antibody-dependent enhancement effect. Antiviral preparation based on plant source has greater potential in combating dengue disease. Investigations on antiviral effects of standardized methanolic extracts of *Andrographis paniculata, Citrus limon, Cymbopogon citratus, Momordica charantia, Ocimum sanctum* and *Pelargonium citrosum* on dengue virus serotype 1 (DENV-1) revealed that *O. sanctum* contained 88.6% of total flavonoids content, the highest among all the six plants tested (Tang Leon *et al,* 2012). The maximum non-toxic dose (MNTD) determined of methanolic extract against Vero E6 cells *in vitro* was in decreasing order of *M. charantia* > *C. limon* > *P. citrosum, O. sanctum* > *A. paniculata* > *C. citratus.* Antiviral assay based on cytopathic effects (CPE) exhibited slight inhibition with methanolic extracts of *O. sanctum* and *C. citratus,* although a significant inhibition was not observed in MTT assay. A higher antiviral inhibitory effect was shown by *A. paniculata* followed by *M. charantia* of the six medicinal plants tested by degree of inhibition on CPE.

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as omeprazoleare prescribed for healing inflammation, fever and pain due to most prevailing gastrointestinal disorder - Peptic Ulcer Disease (PUD) encompassing gastric and duodenal ulcer. Yet, NSAIDs cause gastric damage as a major

adverse reaction. To overcome this impairment a study at Navodaya Medical College and Research Centre for two years on the gastro protective effect aqueous extract of *O. sanctum* was compared with omeprazole in model of indomethacin-induced ulceration. Separate group of rats administered with *O. sanctum* (200 mg/kg or 400 mg/kg) or omeprazole (10 mg/kg) resulted *O. sanctum* with significant ($p < 0.05$) protective effect against indomethacin-induced gastric ulcer when compared to control. The reason quoted for this gastro protective effect of *O. sanctum* was comparable with that of omeprazole due to its 5-lipoxygenase inhibitory effect, mucoprotective activity and its antisecretory effect (Mrutyunjay and Sameer, 2014).

The safe, efficient and inexpensive use of tulsi plant with ease of accessibility has recognized and attributed this plant as equivalent to "matchless one" herb for medicinal and therapeutic use. The chemical constituent in it cures many diseases and relies on antiageing, immunomodulatory including antimicrobial and anticancer properties. Thus, tulsi is named as

Queen of Herbs of India and is one of the holiest and sacred herbs. Traditionally Ayurvedic and Unani systems have vital role and fame for tulsi. A review by Thakur and his group (2017) provides links from several aspects of scientific works on tulsi uses. Its use in Ayurvedic medicine through the extracts are commonly used for headaches, cold, soreness, stomach disorders, heart sickness, malaria and range of poisoning is explained well with evidences.

Of late study on Tulsi herb (Upadhyay *et al.* 2015) reports that the sequencing of draft genome reveals that herb has 374 million bases and the exploration of medicinal properties due to main enzymes coding for the synthesis of secondary metabolites has been already done. Of the 14 metabolites mapped, 8 have anti-cancer properties and the remaining 6 have antifungal, antioxidant, anti-inflammatory, antiseptic and cardio protective properties.

Tulsi, also known as holy basil, is indigenous to the Indian continent and highly revered for its medicinal uses within the Ayurvedic and Siddha medical systems. Many *in vitro*, animal and human studies attest to tulsi having multiple therapeutic actions including adaptogenic, antimicrobial, anti-inflammatory, cardioprotective and immunomodulatory effects. Till date there are no systematic reviews of human research on tulsi's clinical efficacy and safety. A comprehensive literature review of human studies has been reported on a clinical outcome after ingestion of tulsi (Bano *et al*, 2017). The results are outcomes of studies published in books, thesis, conference proceedings, and electronic databases including Cochrane Library, Google Scholar, Embase, Medline, PubMed, ScienceDirect, and IndianMedical databases. Twenty four studies are

been identified that reported therapeutic effects on metabolic disorders, cardiovascular disease, immunity, and neurocognition. All studies reported favourable clinical outcomes with no studies reporting any significant adverse events. The reviewed studies reinforce traditional uses and suggest tulsi is an effective treatment for life style related chronic diseases including diabetes, metabolic syndrome, and psychological stress. Further studies are required to explore mechanisms of action, clarify the dosage and dose form, and determine the populations most likely to benefit from tulsi's therapeutic effects.

The health benefits of holy basil and ways of integrating it into day to day life to feel less anxious and more social is shown by studies (Brian and Ana, 2017). Holy basil has been shown to have antidepressant and antianxiety properties similar to antidepressant drugs. It can be used directly raw, cook with it, supplement with any form or can use as tea and also offered as essential oil.

Healing benefits of tulsi is remembered with rapid home medicines by using leaves and flowers for colds, coughs, infections and fever. The practice of placing a potted tulsi plant in front yard in Indian households very well confirms the germicidal actions of the plant (Desi wisdom, 2018).

The rich medicinal importance of tulsi (*Ocimum sanctum*) is evidenced with the use of leaves and flowers having good nutritional sources of fatty acid, β-carotene, calcium, vitamin C, and volatile substances which maintain our body healthy. Hindus use the wood or seeds of tulsi to make garland, which are strings of beads used to help in meditation, focusing of mind, chanting a devotional practice which connects the body, mind and spirit. The review by Ashok Kumar and coauthors (2018) has focused on the beneficial effects such as medicinal, nutritional and spiritual properties of tulsi.

Among the important medicinal plants, the plants of genus Ocimum from family Lamiaceae are of high potential. Two types of tulsi varieties are reported as purple/black (Krishna Tulsi) and green (Rama Tulsi) share similar chemical constituents. Plants are rich sources of medicine and large drugs in use are derived from plants. The healing uses are economical, safe and effective due to their ease of availability. Sanskrit word tulsi means "matchless one". Numerous medicinal properties are reported about Tulsi plant not only in Ayurveda and Siddha but also in Greek, Roman and Unani systems of medicine (Siyanna, 2018). Abundant medicinal and health benefits of tulsi are been confirmed by scientists. Outcomes of research determine the tulsi plant extract can

treat at least 20 conditions. Thus, Holy Basil (*Ocimum sanctum*) has plentiful uses in medicine. (Case Adams, 2019)

Tulsi Leaf and anti-Cancer benefits

Exhibition of anticancer activity was found in extracts of tulsi leaf extracts tried on tumor cell cultures. The exhibition of anticancer activity of tulsi leaf extracts upon use on tumor cell cultures is reported by Sridevi and coworkers (2016). This cancer-preventive benefit is in fact recognized as eugenol, a potential flavonoid including methanolic extract from tulsi leaf extract. Advantages of tulsi leaf and cancer are reported by using mice instead of humans that the tulsi also benefits against radiation poisoning. This findings support that additionally presence of flavonoids in tulsi is been able to protect normal tissue cells from harmful effects of radiation including anticancer properties (Singh *et al*, 2016). Even though authors emphasize the need of larger trials on humans for promising results to examine the efficiency of benefits of tulsi in individuals. There is an evidence to compare the regular consumption of tulsi tea and yoga practice permitting the practitioner with an overall sense of calmness by Christabel Lobo (2019).

Phytochemical analysis of tulsi

Plant extracts continues the numerous searches for more effective drugs of plant origin which are less toxic and available for low socio-economic population in the treatment of diseases caused by pathogenic bacteria (Verma *et al,* 2019). The qualitative analysis using hydroalcoholic extract by GC-MS for determination of phytochemical screening and probable chemical constituents of extracts from the leaf sample of *Ocimum sanctum* exhibited the identification of 10 phytochemical constituents predominantly eugenol (43.88%) and caryophyllene (26.53%) and other compounds like tannins, saponin, flavonoids, steroid, terpenoids and cardiac glycerides (Devendran and Balasubramanian, 2011). Study also reported the absence of phlobatannin. The major components present in leaves of *Ocimum sanctum* were Eugenol, Caryophyllene, Cyclopentane, Cyclopropylidene (1.02%), Cyclohexane, 1,2,4-triethenyl (15.31%), octadecane, 1,1-dimethoxy (2.04%) and Benzene methanamine, N,N,a,4-tetramethyl-(2.04%). These phytochemical constituents contribute to the medicinal activity of the hydroalcoholic extract of *Ocimum sanctum*. The presence of eugenol and caryophyllene in the leaves is considered mainly responsible for various antimicrobial properties. Eugenol in its major presence is responsible for its repellent,

antioxidative property and also supposed to be responsible for inhibition of lipid peroxidation. Thus contributing for achieving good health by preventing heart diseases chances as well as most of the other biochemical diseases since oxidative stress is the symbol of such diseases.

The quantitative analysis of dried powder of tulsi using soxhlet apparatus individually with methanol, ethanol and distilled water separation presents several secondary metabolites such as carbohydrate, tannin, flavonoids, saponins, glycoside, terpenoid, fatty acids and phenol. High amount of phenols were documented from 1.6 – 7.6 %, alkaloid and flavonoids were 0.91 to 1.28 % and 1.56 to 2.24 %. GC-MS analysis of methanolic extract identified compounds namely Eugenol, Benzene, 1, 2-dimethoxy- 4-(2- propenyl), α - Farnesene and Cyclohexane, 1, 2, 4-triethenyl as chief constituents (Borah and Biswas, 2018). Presence of these phyto-chemicals are known to possess antiseptic, analgesic, anti-inflammatory, antimicrobial, antistress, immunomodulatory, hypoglycemic, hypotensive and antioxidant properties. Henceforth, it is more advantageous to practice tulsi as an herbal medicine compared to any chemically synthesized drug.

The first report from under-explored camphor tulsi of genus Ocimum was on any gene of MVA/isoprenoid pathway to provide deep insight into OkHMGR for regulation of biosynthesis of non-plastidal isoprenoids (Shilpi *et al.* 2018). This study suggests that OkHMGR might be a potential tool for attempting metabolic engineering for enhancing medicinally important terpenoidal metabolites in plants. In essential oil from tulsi, the presence of terpenoids is unique feature of *Ocimum kilimandscharicum* unlike other *Ocimum species* are renowned for phenylpropanoids as major constituents. The cloning of 1.7-KbORF encoding ~60-kDa protein responsible for key enzyme of MVA/terpenoid metabolic pathway *viz* 3-hydroxy-3-methylglutaryl Co-A reductase (OkHMGR) was done from *O. kilimandscharicum* (Shilpi *et al.* 2018). The kinetic characteristics of this protein revealed the availability of HMG-CoA as a regulator point of MVA-pathway. The tissue-specific functions of this gene in flower and leaf tissue with accumulation of terpenoidal essential oil was interpreted by Transcript profiling of the OkHMGR. The differential regulation of OkHMGR in response to exposure methyl jasmonate, salicylic-acid, and stress conditions such-as salt and temperature stress demonstrated its pivotal role in managing signaling and stress-responses. The transient over-expression study of OkHMGR in both homologous and heterologous plants such as *O. sanctum, O. basilicum, O. gratissimum*, *Withania somnifera* and *Artemisia annua* revealed that the additional OkHMGR in-planta could afford endogenous flow of isoprenoid units towards synthesis of terpenoids.

A study on crude extracts of *Ocimum tenuiflorum* L. leaves for phytochemical screening assay with methanol fractions performed did notshow any steroid and terpenoid (Leila *et al*, 2018). Even ethanol and butanol fractions also did not exhibit steroid in the screening test. The HPLC identification results of active crude extracts and active fractions displayed the possiblebioactive compounds which may control diabetes. The presence of polyphenolic active constituents such as 3,4-dimethoxycinnamic acid, caffeic acid, diosmetin, luteolin, kaempferol, rosmarinic acid, apigenin and genistein in these extracts controlled the blood glucose levels in diabetic rats. This study suggests that furthermore detailed isolation of the active components could pave the way for the development of new agents for the treatment of diabetes and its complications.

A demonstration study on the phytochemical analysis of methanol and aqueous extracts of *O. sanctum, A. indica* and *P. emblica* for developing antibacterial compound as phytomedicine to act against microbes (Verma *et al,* 2019). The study concentrated on the presence of phytoconstituents like tannin, saponins, flavonoids, glycosides, reducing sugar, steroids and alkaloids. The intention of the study was to develop plant based antibacterials with enormous therapeutic potentials and lesser side effects that are often associated with synthetic antibacterials.

Animal nutrition supplement studies in Tulsi

A study conducted at Bangaldesh on determination of efficacy of tulsi and ginger as growth promoter in broilers concludes that using herbal extract is profitable and suitable for human consumption (Wasim *et al.* 2018). The gain in net body weight of thirty day old chicks after consecutively 5 weeks after 7th day of experiment with tulsi and ginger solution, with tulsi solution @ 1ml/litre and with ginger solution @ 1 ml/litre was 1393±11.07, 1533±11.98 and 1588±12.10 gm respectively. The results were in spite of not practicing vaccination and antibiotics schedule in ration. The net profit per broiler recorded was 18.82, 36.13 and 42.53 Tk respectively confirming the use of herbal extract for economic, profitable and safe production of broiler for human consumption.

Biotechnological studies in Tulsi

Molecular marker studies in Tulsi

Molecular marker studies provides more detailed molecular level of information on the characterization, genetic diversity, genetic relatedness among the genotypes working with the use of molecular marker aided tools available in the *Ocimum species*. Although efforts are been made in this area of work, there is a lack of information on the molecular characterization of *Ocimum species*. Few of the studies reported predominantly are been presented below.

Molecular characterization of 17 Ocimum genotypes belonging to 5 different species (*O. basilicum*, *O. americanum, O. sanctum, O. gratissimum* and *O. polystachyon*) was attempted through Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) markers (Hardik *et al.* 2015). The investigation results presented suitable guidelines for collection, conservation and characterization of Ocimum genetic resources with the observation of maximum number of unique alleles in *Ocimum sanctum*. Highly polymorphic loci (98.17 %) with 234 of 238 loci was achieved through ISSR primers compared to RAPD primers (96.47 % polymorphic) with 490 of 506 loci (20 RAPD primers) using PCR amplification. The average PIC value for RAPD ranged was 0.937 in contrast to ISSR was 0.923. The average of Jaccard's similarity coefficient based on RAPD and ISSR analysis was 0.58 and 0.52, respectively. Clustering pattern of dendrogram generated using the pooled RAPD and ISSR data showed all Ocimum genotypes in their respective species groups at a cutoff value of 0.49 and 0.42, respectively. Many unique species specific alleles were amplified by RAPD and ISSR markers.

A greater variability is displayed in the genus Ocimum, (family Lamiaceae). Owing to the prevalence of cross pollination, interspecific hybridization and polyploidy has complicated its taxonomy and made difficulty in studying systematics. A study on investigation of morphological and biochemical variability among 18 ecotypes collected from different parts of the Assam state, India has found considerable diversity using all approaches (Ranju *et al.* 2016). Wide variability was reported with quantitative morphological parameters and was not correlating often with their geographical distances including biochemical parameters. The range of Euclidean distance was 6.06 between the closest accessions and 59.00 between the most distant accessions. The accessions were divided through phylogenetic analysis into two major clusters (approximately 76% identity) and a minor cluster (with three accessions) sharing only approx. 65% identity with the other two. Greater diversity was observed in qualitative characters also which divided ecotypes into two to five groups. Wide polymorphism was exhibited among biochemical characters with distance range from

1.690 to 58.574. The accessions divided into two major clusters through phylogenetic mapping involving approx. 80% similarity. This study stresses the requirement of further correlation of distance based on both morphological and biochemical characters.

Essential oils from different *Ocimum species* show significant variations in content and composition based on their distinct morphological characters and harvesting seasons. A comparison study on various germplasms of *Ocimum basilicum* grown in India for essential oil content and composition was tried to detect the superior germplasm rich in desirable chemical components, such as methyl chavicol and eugenol (Parmeshwar *et al,* 2017). The idea was to utilize these chemical components for commercial cultivation in western plains of India. Chemical characterization of hydro distillation extracted essential oils was done on Gas Chromatograph–mass spectrophotometer (GC–MS). Leaf colour and panicle type varied from green to purple green and bunchy, individual and intermediate type. Oil percent was maximum in DOB-1 (0.70%), followed by DOB-5 (0.61%) at stage II. Higher essential oil yield was found in leaves harvested at stage II than stage I. Methyl chavicol content increased at full flowering (stage II), whereas Eugenol and methyl eugenol content increased at onset of flowering (stage I). Only DOB-4 was rich in methyl cinnamate otherwise majority of the germplasm were reported to be rich in methyl chavicol. Positive correlations are observed in the intensity of purple colour of leaf with lines rich in methyl chavicol. Relationship between maximum methyl cinnamate (DOB-4) content, average minimum plant height and leaf size were positive. The authors alert that it is important for crop improvement programme to understand these key factors in field and information on superior lines DOB-1 and DOB-5.

Determination of diversity of nine Ocimum genotypes naturally grown in the Dakshin Dinajpur district of West Bengal, India has been attempted to know the level of variation in Ocimum genus based on morphological, chemical and randomly amplified polymorphic DNA (RAPD) (Tanmay *et al,* 2017). Of the nine Ocimum genotypes tested six (*O. americanum, O. africanum, O. basilicum, O. gratissimum, O. kilimandscharicum* and *O. tenuiflorum*) were different species while rest are varieties. Evaluation of 18 qualitative and 17 quantitative morphological traits and chemical compositions are reported. Morphological traits exhibited significant variations except in two species (*O. africanum* and *O. basilicum*) and morphological data cluster displayed two different groups (basilicum group and sanctum). No much variation was reported by chemical analysis between morphologically similar species *viz. O. africanum* and *O. basilicum*, whereas differences

were indicated clearly between them through RAPD analysis. Thus, the study confirms that the delineation of taxonomic confirmation approach is best possible when combined analysis of morphological traits, chemical and molecular markers is done. Also *O. africanum* is been reported first time from West Bengal, India.

Genome sequencing studies in Tulsi

Whole genome sequencing of two different varieties of the sacred Indian plant - Holy basil plant tulsi was reported in 2015 by two separate groups of genomic scientists – one from the CSIR-Central Institute of Medicinal & Aromatic Plants (CSIR-CIMAP), Lucknow and another from the National Centre for Biological Sciences (NCBS), Bangalore. In the same year (June 2015) CSIR-CIMAP scientists published the whole genome sequence of *Ocimum sanctum*, which is worshipped by Hindus as 'Vishnupriya', while in September 2015 - NCBS scientists produced the first draft genome of *O. tenuiflorum* or the 'Krishna' subtype. Both the genomes kept open the immense medical, metabolic and therapeutic potential of the much revered herb described in ancient Indian Ayurvedic text Charaka Samhita as the "Queen of herbs".

Ocimum varieties are widely used in traditional Greek, Roman, Siddha and Unani systems of medicine. Tulsi is known for their therapeutic roles due to its richness in phenylpropanoids, terpenoids and their derivatives. The whole genome sequence information is not only useful for identifying genes involved in production of therapeutic molecules, but also helps in several applications like facilitation of identifying genes which are not identified yet in synthesis of important secondary metabolites in this plant and utilization of genomic data for production of secondary metabolites through synthetic biology approaches. By comparing the genomic data the scientists revealed that the maximum evolutionary closeness of *O. sanctum* to *Salvia miltiorrhiza*, a traditional medicinal plant used in Chinese medicine system (Subhra, 2015).

Domesticated tulsi subtypes contain very high concentration of medicinally important metabolites. This was the first time, a draft genome covering 374 million bases (61 per cent coverage) of the tulsi herb has been sequenced and the main enzymes responsible for the synthesis of secondary metabolites which have medicinal properties have been unravelled by a team of nearly 30 scientists at the Bengaluru-based National Centre for Biological Sciences (NCBS) and the Centre for Cellular and Molecular Platforms, Bengaluru. Tulsi is well known for its myriad medicinal properties like

antibacterial, antifungal, antipyretic, antioxidant, antiseptic and anticancer. Of the nearly 40 secondary metabolites that have medicinal value, the genes and enzymes responsible for the production of 14 metabolites have been mapped on the genome. Not much information on the pathway is available for the remaining metabolites. Of the 14 metabolites that have been mapped, eight have anti-cancer properties and the remaining six have antifungal, antioxidant, anti-inflammatory, antiseptic, and cardio protective properties.

It is known that tulsi contains many anticancer and other medicinally important properties. As evidence to this, only two domesticated subtypes - Rama (green leaves) and Krishna (purple leaves) out of the five tulsi subtypes investigated exhibited very high concentration of these medicinally important metabolites. Of these two domesticated subtypes, the Rama subtype contains "high abundance" of medicinally important metabolites. The concentration of eugenol (anti-infective) and ursolic acid (anticancer) in both leaf and stem was more in Rama than Krishna subtype (Upadhyay *et al*, 2015). Of the various parts of the plant, the young leaves were found to have the highest concentration of the metabolites; roots and stems contain only minimal amount. The reason for the abundant presence of the metabolites in leaves could be that they are the source of energy production and they are most vulnerable to consumption by animals and exposure to pathogens (Prasad, 2015).

The draft genome of *Ocimum tenuiflurum* L (subtype Krishna Tulsi) is reported by Atul K Upadhyay and coworkers (2015). Information on whole genome sequencing was generated using paired-end and mate-pair sequence libraries withIllumina Hiseq 1000 with an assembled genome size of 374 Mb covering of 61 % (612 Mb estimated genome size) of genome. Transcriptomes (RNA-Seq) study of Krishna and Rama Tulsi (subtypes of *O. tenuiflorum*) with relative expression of genes in both the varieties has been reported. A detailed study of pathways for production of medicinally-important specialized metabolites in comparison with parallel pathways in *Arabidopsis thaliana* and other plants is also been done. Relatively high expression levels of anthocyanin biosynthesis-related genes in leaf samples of Krishna Tulsi are presented supporting the purple colouration of Krishna Tulsi leaves. Validation study by q-RT-PCR from different tissues of five different species confirmed the expression of six important genes identified from genome data. This study displayed the high extent of urosolic acid-producing genes in young leaves of the Rama subtype. These results confirm that these are potential drugs in curing several ailments including cancer using mass spectrometry due to the presence of eugenol and ursolic acid. The study group

has also indicated that the whole genome sequence information availability of *O. tenuiflorum* and its analysis with small changes in amino acid at functional sites of genes underlying metabolite synthesis pathways can impart special medicinal properties to this herb.

Bioinformatics studies in Tulsi

Holy basil or tulsi- *Ocimum tenuiflorum*, is a globally recognized medicinal plant with assembly of medicinal properties. Although, there is a gap in revealing the complex interplay among its constituent proteins at subcellular level with comprehensive study. In order to bridge this gap, TulsiPIN, a genome scale interologous protein-protein interaction (PPI) network is developed using 49 template plants (Vikram Singh*et al,* 2019). The reported network consists of 13; 660 nodes and 327; 409 binary interactions. A high confidence PPI network consisting of 7; 719 nodes having 95; 532 interactions was inferred using domain-domain interaction information along with interolog based statistics, and its reliability was further assessed using functional homogeneity and protein colocalization. 1; 625 vital proteins are predicted by statistically evaluating this high confidence TulsiPIN with two ensembles of corresponding random networks, each consisting of 10,000 realizations of Erdos-Rényi and Barabási-Albert models. Topological features of TulsiPIN including small-world, scale-free and modular architecture are inspected and found to resemble with other plant PPI networks. Finally, numerous regulatory proteins like transcription factors, transcription regulators and protein kinases are profiled in TulsiPIN and a subnetwork of proteins participating in 10 secondary metabolite biosynthetic pathways is studied. The methodology developed and insights imparted would be usefulin understanding regulatory mechanisms in various plant species.

Nanotechnological studies in Tulsi

The field of nanotechnology is the most active area of research in modern materials science. Though there are many chemical as well as physical methods, green synthesis of nanomaterials is the most emerging method of synthesis. Researchers have developed a new kind of antibiotic cotton by coating the fabric with nanoparticles loaded with leaf extracts of India basil 'tulsi' (*Ocimum sanctum*). Cotton fabrics coated with tulsi-loaded sodium alginate chitosan nanoparticles stifle the growth of disease-causing bacteria and fungi. Such fabrics could be used to make scrubs to check the spread of infectious diseases. A methanol-based extract proved most effective in inhibiting the

growth of four bacteria *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and two fungi *Aspergillus niger* and *Penicillium sp.* There was a 100% inhibition of all test microorganisms except *E. coli* with leaf extract-loaded nanoparticle-coated cotton fabrics. The retention of antibacterial activity after 20 washes was reported by laundry tests in coated cotton fabrics. The presence of antimicrobial compounds in leaf extracts were cited by researchers as attached to fiber surface by forming bonds. The compounds then permeate and rupture the cell walls of microorganisms, resulting in leakage of cellular metabolites. This stops the reproduction of the microorganisms. The authors of this work are from: PG & Research Department of Microbiology, PSG College of Arts & Science, Coimbatore, Tamil Nadu & Defence Bioengineering and Electromedical Laboratory, Bangalore, Karnataka, India; and Budapest University of Technology and Economics, Hungary. DOI:10.1038/nindia.2012.132 published online 14 September 2012 Nature India EISSN: 1755-3180.

Synthesis of antibacterial silver nanoparticles (AgNPs) using leaf broth of medicinal herb, *Ocimum sanctum* (Tulsi) has been attempted by Charusheela and team (2013). The characterization of synthesized AgNPs done by UV-Vis spectroscopy, transmission electron microscopy (TEM), and X-ray diffractometry. TEM results confirmed that the mean particle of synthesized NPs was found to be 18 nm. The qualitative assessment of reducing potential of leaf extract has also been carried out which indicated presence of significant amount of reducing entities. FTIR analysis revealed that the AgNPs were stabilized by eugenols, terpenes, and other aromatic compounds present in the extract. Such AgNPs stabilized by Tulsi leaf extract were found to have enhanced antimicrobial activity against well-known pathogenic strains, namely *Staphylococcus aureus* and *E. coli*.

Synthesis of SNPs (Silver Nanoparticles) or (Green-Silver) in aqueous medium using leaf extracts of *Ocimum sanctum* L was studied by Bindhani and Panigrahi (2015). The characterization of synthesized silver nanoparticles (SNPs) was done by the different techniques such as UV-vis Spectrophotometer, XRD, FTIR and TEM. The synthesis of silver nanoparticles were confirmed due to the SPR optical absorption band peak at ~440 nm by UV-vis spectrophotometer, nearly 15-45 nm in diameter with spherical in shape by TEM, high crystalline with the Bragg peaks of (111), (200), (220) and (311) plane as the predominant orientation by XRD and FTIR confirmed the presence of methoxy and allyl groups in SNPs. The reduction of Ag metal ions was performed due to the presence of various types of anti-oxidants in the leaves of *Ocimum sanctum* L. and each having a unique structure and functions. The formations of silver nanoparticles through aqueous

medium were fairly stable and which can be incubated up to 4 months in refrigerator. At last, the antimicrobial activities of AgNPs were evaluated using different bacteria strain. It was found that, this biosynthesizing of silver nanoparticles may inhibit the all types of bacterial pathogenic organisms competently. This recent research study opens an innovative design to progress our understanding of how SNPs behave can be optimized to improve human antimicrobial activities.

The most effectively studied nanoparticles in the current past are those made from the noble metals such as silver, gold and platinum. Nanoparticles find vast applications in various fields ranging from medical to physical fields. To meet the increasing demands for commercial nanoparticles new eco-friendly "green" methods of synthesis are being discovered. Plant mediated synthesis of nanoparticles offers single step, easy extracellular synthesis of nanoparticles. The leaf extract of *Ocimum sanctum* was used as a reducing agent for the synthesis of platinum nanoparticles from an aqueous chloroplatinic acid (H2PtCl6·6H2O). A greater conversion of platinum ions to nanoparticles was achieved by employing a Tulsi leaf broth with a reaction temperature of 100°C. The successful formation of platinum nanoparticles has been confirmed by UV-Vis spectrophotometer, Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) (Prabhu *et al*, 2017). It is evident from scanning electron microscopy that the reduced platinum particles were found as aggregates with irregular shape. FTIR studies confirmed the presence of platinum nanoparticles which may responsible for reduction of platinum ions to platinum nanoparticles, yet further research is needed in this area to explore the possible biomolecules responsible for the bioreduction process. The XRD pattern revealed the complex crystalline nature of platinum nanoparticle. In this method there is no need to use high pressure, energy, temperature and toxic chemicals in case of chemical and physical method.

Conclusion

With the available information and evidences from the above studies and results it is very much certain that tulsi is truly all time beneficial and medicinal plant for not only common day to day aliments, but also can cure cancer like diseases which are challenge for humankind. Although the information provided here may serve as reconfirmation of the curative ability of the medicinal herb tulsi, further studies on this sacred plant may be required to dissect more pathways underlying the medicinal values using the latest tools and technology available.

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