Discussion

Aromatic plants and fragrant flowers are some of nature’s most beautiful creations. In the long history of planetary evolution it was the appearance of flowers that initiated the rapid expansion of biodiversity that has created the world we now live in. For global benefit, medicinal plants, especially the aromatic species are the key in solving numerous interrelated global issues. Phytochemicals are being used from ancient time. There are many plants which are used as herbal medicine on account of their phytochemicals. Considering medicinal value of Tulsi (*Ocimum sanctum* L.) present studies have been taken. These studies, though are quite exhaustive, yet demands critical analysis, for this purpose chapter wise discussion is being given here. Gislene (2000) prepared the ethanolic extracts from the plants were utilized: *Achillea millifolium* (yarrow), *Caryophyllus aromaticus* (clove), *Melissa officinalis* (lemon-balm), *Ocimum basilicum* (basil), *Psidium guajava* (guava), *Punica granatum* (pomegranate), *Rosmarinus officinalis* (rosemary), *Salvia officinalis* (sage), *Syzygyum joabolanum* (jambolan) and *Thymus vulgaris* (thyme) plants. Halim (2006) prepared the aqueous extract of *Ocimum sanctum* (Tulsi) leaves. Deepthi (2007) observed the effect of plant extract and Acetone precipitated proteins from six medicinal plants against *Tobamovirus* infection. However, she did not prepare the aqueous extract. Bhushan Bhaskarwar (2008)
worked on antimicrobial activity of *Jatropha podagrica* with plant extract. Pranay Jain (2009) also prepared aqueous leaf extracts of different plants such as *Mentha arvensis* (Mint), *Curcuma longa* (Turmeric), *Piper nigrum* (Black pepper), *Zingiber officinale* (ginger) and *Azadirachta indica* (Neem). Shankar (2009) prepared the essential oil from the alcoholic extract of leaves and fixed oil from seeds of *Ocimum*. Jana (2010) worked on phytochemical analysis and antibacterial screening *in vivo* and *in vitro* by ethanolic extracts of Indian medicinal herb: *Anethum graveolens*. Ajayi (2010) prepared the leaves extract of *C. occidentalis*, *C. zambesicus* and *N. laevis*.

For the present study five types of extracts were used for estimation of pH. These are aqueous extract, acetone extract, extract (steam distillation), pure eugenol and crude extract. The pH of these extract were detected as 7, 6, 6.5, 5 and 6 respectively. Earlier authors not mentioned pH of their extract of medicinal plants.

Protein estimation in the Tulsi extracts was made by Lowry Assay. The O.D were taken for the protein estimation by Photocalorimeter. The data showed that in the Tulsi leaves 32-50 µg/mL protein is present. The extract number one showed maximum 50 ± 0.5 µg/mL protein. Extract no 2 showed 42 ± 0.5 µg/mL protein. Extract no 3 showed 32 ± 0.5 µg/mL protein in the leaves. The extract no 4 and 5 did not exhibit any protein. The data are supported by the work of Suresh (1992), Kothari (2004), Shokeen (2008) and Sankaran Nair.(2010) on the medicinal plants.

The result of scanning absorption spectrum showed λmax is 321-360nm. The pure eugenol λmax is 338nm. It is near to the
λ_{max} 338nm to show the presence of eugenol as main constituent. The structure of the eugenol is determined with mass spectrum peak and IR Spectroscopy. Identification of compounds was made on the basis of retention indices of the peaks values determined in Wiley and NBS libreries spectra literature. Tulsi (Ocimum sanctum L.) leaves has 3%-5% eugenol. The work is supported by the findings of Brophy (1993), Machado (2002), Kothari (2004), Kothari (2005). Sunita Bansod (2008) said that the eugenol is the main constituent of *Ocimum sanctum*. However, she did not determine the structure of eugenol as well as not mentioned the percentage of the eugenol oil in the Tulsi leaves.

Bruce (1986) studied the polypeptide by SDS gel electrophorisis method from membrane fractions of the hepatocyte, which is specifically associated with the tight junction in the rat tissue. Andre (1991) studied Anomalous behaviour of a protein during SDS/PAGE corrected by chemical modification of carboxylic groups. Maizel (2000) performed an excellent discussion of the immediately obvious success of SDS PAGE over the older tube gel method by early experimenters with SDS method.Lay-Harn (2005) find out the SDS-PAGE Electrophoretic property of human chorionic gonadotropin (hCG) and its β-subunit. In the present study in the Tulsi (*Ocimum sanctum*) leaves 97.4 kDa molecular wt protein has been estimated by this method. No attempt was made yet, to determine the Tulsi protein by SDS gel electrophoresis method.

Eugenol is the main constituent of the Tulsi leaves. Only few researchers described the eugenol properties extracted by other
medicinal plants. In the present thesis detailed chemical studies has been described of the eugenol extracted by Tulsi (*Ocimum sanctum* L.) leaves. These chemical properties are also supported by the work of Pramod (2008) and Neuenschwander (2010).

*Salmonella typhi* and *Escherichia coli* are facultative intracellular pathogens. These are obligate anarobes bacteria. Antibiotics used for controlling these sometimes interact with other drugs, raising or covering sercum levels of other drugs by increasing or decreasing the metabolic activity. The bactericidal drugs kill bacteria, slow or stop in vitro bacterial growth. It also affect the human health by their side effects. For the present study, these bacteria (*Salmonella typhi* and *Escherichia coli*) samples were collected from the human as well as from pathological lab. and cultured in the laboratories on the culture media in plates. The serial dilutions of the extract were made and these dilutions were poured in the bacterial plates to see antibacterial activities. The results showed that the pure eugenol has the maximum antibacterial activity against both *Salmonella typhi* and *Escherichia coli* in comparison to raw extract. It has the zone of inhibition with eugenol 25mm as comparable with Ampicillin 14mm and acetone as control 8mm.

The higher activity of extract can be explained on the basis of the chemical structure of their major constituents such as dill-apiole and anethole, which have aromatic nucleus containing polar functional group that is known to form hydrogen bonds with active sites of the target enzyme. Saxena *et al.* (1994) documented a MIC varying from 12.5 to 1,000 µg/mL when testing different
concentrations of Rhus glaba extracts on both, gram-negative and gram-positive bacteria. The results revealed variability in the inhibitory concentrations of each extract for given bacteria. Gislene et al., (2000) said that extracts from jambolan and clove showed activities in the range (concentrations) from 50 to 500 µg/mL, and from 20 to 250 µg/mL, respectively. The lowest variation was observed for eugenol, perhaps due to its purity. It is reported that its seed oil has a little activity against gram-negative bacteria, which may be due to the differences in composition related to variety; agronomic practice and processing which also influence concentrations of active ingredients, hence, effecting antimicrobial properties (Delaquis et al., 2002). Same antibacterial activity was reported by Pradeep and Bhora (2004) but using different plant Actieneopteris radiate. As depicted from the results of antibacterial activity against all strains, ethanolic seed extract and aqueous extract have shown maximum activities. Hence, seeds have more potential than other plant parts in terms of microbial activity. It may be due to the constituents present in the seeds of Anethum graveolens, it is rich in carvone (55.2%), limonene (16.6%), dill-apiole (43.2%), linoleic acid (23.1%) and anethole (11%) (Singh et al., 2005). Similarly, acetone extract of Anethum graveolens and its seed oil has been reported to show statistically significant antibacterial activities against Staphylococcus aureus, Bacillus cereus, Salmonella typhii, Pseudomonas aeruginosa, Bacillus subtilis as well as antifungal activities against Aspergillus niger, Fusarium moniliforme, Penicilium citrinum. The antibacterial susceptibility test showed that the ethanolic extracts of both plants
has higher inhibition on all the test isolates giving a zone of inhibition with diameter range of 1.0±0.71 to 9.3±0.63mm as compared to the aqueous extract with low inhibition activity of 1.0±0.71 to 5.7±1.2mm. The high activity of the ethanolic extracts verifies the use of the ethanolic extraction method by local herbalists (Allero and Afolayan, 2006).

*Anethum graveolens* have been in use for many years as decoctions or infusions prepared in water to treat ailments. It has been reported that aqueous extracts of *Anethum graveolens* showed a broad-spectrum antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella flexneri* and *Salmonella typhii* (Arora and Kaur, 2007). Deepthi 2007 prepared the acetone extract of six plant to seeing the effect against Tobamovirus infection. In the present study the acetone extract was tested against the *salmonella typhi* and *E. coli* pathogens.

Bhushan Bhaskarwar *et al* (2008) worked on the different plant extract with some bacterial strain and find out the evaluation of antimicrobial activity of medicinal plant *Jatropha podagrica* (Hook). He prepared the hexane extract of stem bark and found to be active on most of clinical isolates of *S. aureus*, *E.coli* and *Candida albicans*. Phytochemical test confirms the presence of steroids and triterpenes. In most cases 15mg/ml concentration shows the maximum activity, which revealed *Jatropha podagrica* as novel antimicrobial agent. However, he did not find out the zone of inhabitation of the bacterial strains. Omwenga *et al* (2009) observed less antibacterial activity of the methanol extract of
*Boscia angustifolia.* Deepak Sharma (2009) studied comparative screening of antibacterial activity of weed extract *Dathura stromonius* and *Azadiracta indica.*

Similarly, Ajayi and Akintola (2010) showed that most of the plants extracts (*Cassia occidentalis, Croton zambensis* and *Newbouldia*) were less effective against the strain of *E. coli* tested except, *C. zambesicus* which had the highest antibacterial inhibition zone of 2 mm. Oluduro and Omoboye (2010) showed that all the plants parts studied possess antimicrobial properties, with greater antimicrobial efficacy when used synergistically. This might be due to the resultant effect of the active agents in the plant materials. The antibacterial activities of all the extracts of the plants materials either when used separately or combined were concentration dependent as zone of growth inhibition increased with increasing concentration of the extracts. Jana (2010) used the ethonlic extract of the plant and observed more effective against the *Salmonella typhi* as compare to aqueous extract. In present study, also, acetone extract is observed more effective than the aqueous extract against the *Salmonella typhi.* The Tulsi extract with acetone, crude extract, steam distillation extract and pure eugenol have been observed having good antibacterial activity against *E. coli* and *S. typhi.* with zone of inhibition 05mm – 24mm.

Fungal infection on skin is a serious disease which is caused by *Tinea mycilium.* Its patients number increases during the months of May to September. The crude extract as well as pure eugenol were used for the antifungal activity against *Tinea.* 20% eugenol ointment (EO) was made in the petroleum jelly. Application of EO
was applied on the infected skin for 6-25 days completely cured the ringworm. The major *Tineasis* was completely gone in 20-45 days. The crude extract of Tulsi (*Ocimum sanctum*) leaves also found affective like pure eugenol but treatment needed comparatively more time. Oxenham *et. al*, (2005) reported the fungal activity against mycelial growth of the plant pathogenic fungus *Botrytis fabae*. He applied the methyl chavicol, chematype oil and the linalool chemotype oil against the plant fungus. He also used to reduced the plant fungus infection. He reported the most effective control of fungus infection achived if the treatments were applied 3 hrs. post inoculation.

Lemos *et. al*, (2005) prepared the leaves extract of *Ocimum gratissimum* and used against *Cryptococcus neoformans* skin infection. He observed that *Cryptococcus neoformans* inhibited 23 isolates (92%) of *Cryptococcus neoformans* at a concentration of 62.5 µg/ml while eugenol inhibited 4 isolates (16%) at a concentration of 0.9 µg/ml. In the present study, the 20% eugenol ointment (EO) was observed affective against the *Tineasis*. which (EO) cured Tinea in 6-40 days. Silva *et. al*, (2005) also reported the antifungal activity of *Ocimum gratissimum* towards dermatophytes. Terezinha (2006) investigate the antifungal activity of essential oil obtained by steam distillation (1.1 % w/w) of the arial part of *Ocimum gratissimum*. In the present study the acetone extract was used and this extract had shown good antifungal property against ringworm infection. Sunita Bansod (2008) also used LB Agar disc method to find out the antifungal and antibacterial activity against *Aspergillus fumigatus* niger of 17 plants oils (Including *O.*
sanctum). Koffi koba et. al, (2009) reported the antifungal activity of the essential oils from *Ocimum gratissimum* L. grown in Togo. He recorded minimum inhibitory concentration (MIC) ranging from 300 µl.l⁻¹ to 500 µl.l⁻¹, 500 to 700 µl.l⁻¹ and from 250 to 300 µl.l⁻¹, respectively on dermatophytes, imperfect filamentous fungi and pathogenic yeasts. Deepak Sharma (2009) also studied the antifungal activity with different fungal strain. Amadi et. al, (2010) observed the antifungal activity of *Ocimum gratissimum* extract against *Aspergillus sepens, Curoularic lunata* and *Fusarium monififorme*.

Thus, various authors used plant extracts for curing different fungal infection in plants or animals. Tulsi leaves extract or isolated eugenol oil application bears promising potential for the complete cure of Tineasis in man.

Repellency against house flies revealed that in first hr. 58-67 house flies migrated in untreated part of wooden box. In second hr. 28-35 flies moved in untreated part while in third hr. 3-14 flies shifted in the untreated part. Thus, as a total after 3rd hr. 99-100 % repellency was shown by eugenol oil. On the other hand, insecticides as residual sprays may pose a problem for children and may lead invisible poisoning. Baiting with safer chemicals/materials is a choice for abatement of house fly (Crespo et al., 1998; Hogsette et al., 2002). Jerome et. al, (2002) worked on house flies and tell that boric acid inhibit the development of the House Flies. In the present study the pure eugenol showed 100% repellency against house flies as said above. Sohail et. al, (2005) used non-insecticide baits for households as an important
component of IPM for house fly, because house fly has ability to resist chemicals tested for routine toxicological studies. Further investigations are needed to improve and add the materials readily available for the environmental safer control of house fly.

Only few attempts have been made so far, to observe repellent properties of some phytochemicals against mosquitoes and house flies. Experimental data for present studies for repellency properties of *Ocimum sanctum* reveled that after three hrs. 98-100 percent *Culex* mosquitoes were repelled by crude extract spray in treated part. The same experiment was repeated with eugenol oil which indicates the 100 percent repellent property for *Culex* and *Anopheles* both. With crude extract of Tulsi leaves, 96 to 100% larvicidal activities were recorded for the larvae of (mixed population) *Culex* and *Anopheles* both. Mosquitoes net impregnated with eugenol oil used during nights showed almost cent present repellency. Only in few instances single mosquitoes came near the net and at once took flight away. Thus, both crude extract as well as eugenol oil of Tulsi leaves are found good repellent and larvicide against mosquitoes and their larvae. Moreover, Tulsi leaves extract either in crude form or pure form is ecofriendly and does not cause any pollution hazard as well as ill effect for human being. 20% eugenol based chemical vaporizer envelopes room with its fragrance, repels mosquitoes, driving them outside the living environment. Present studies are supported by the work of Sukumar *et. al*, (1991) on phytochemicals use against mosquitoes as repellent. Bhatnagar *et. al*, (1993) find out the insecticidal properties of essential oils and
major constituents of aromatic plants, *Ocimum basilicum* Linnaeus and *O. sanctum* Linnaeus evaluated against *Anopheles stephensi* Liston, *Aedes aegypti* Linnaeus and *Culex quinquefasciatus* (Say) mosquito species under laboratory conditions. The bioassay tests revealed that the essential oil of *O. basilicum* and its major constituent, methyl chavicol are more effective as compared to *O. sanctum*. Dosages of 0.003 ml/43.0 cm² of essential oil and 0.001 ml/43.0 cm² of methyl chavicol extracted from *O. basilicum* induced 100 per cent mortality in all the three mosquito species in a period ranging from 10 to 25 minutes. Mandavgance *et. al*, (2005) prepared the cow dung based herbal mosquito repellent. In present study the eugenol ointment (EO) and evaporator were made for mosquitoes and found good repellent. Further, investigations are needed to improve and add the materials readily available for the environmental safer control mosquitoes. Anees (2008) used the acetone, chloroform, ethyl acetate, hexane, and methanol leaf and flower extracts of *Ocimum sanctum* against fourth instar larvae of *Aedes aegypti* and *Culex quinquefasciatus*. The highest larval mortality was found in leaf extract of *O. sanctum* against the larvae of *A. aegypti* and *C. quinquefasciatus*. Knio *et al.* (2008) found out Larvicidal activity of essential oils extracted from commonly used herbs in Lebanon against the seaside mosquito, *Ochlerotatus caspius*. Kamaraj *et. al.*, (2008) also supported with different plant extract observed screening for antifeedant and larvicidal activity of plant extracts against *Helicoverpa armigera* (Hübner), *Sylepta derogata* (F.) and *Anopheles stephensi* (Liston). Jerome *et. al.*, (2010) worked on house
flies and said that boric acid inhibit the development of the House Flies. Thus, there is slight variation in repellent and larvicidal properties of different phytochemicals used against mosquitoes, house flies and other insects. In the present study the new phytochemical was introduced as β-sitosterol-D-glycoside. It is supported by Misanur Rahman (2009).

Further, the hemolytic assay proved that the protein present in the extract of Tulsi leaves was not allergic to human as there was no hemolysis in the RBCs.

Thus, present findings on the Tulsi leaves phytochemicals and their use as antifungal, antibacterial and insect repellent are new and will arm the pharmaceutical companies to use them for human welfare for controlling various ailments and nuisance creatures such as mosquitoes and house flies. These studies also open the door for further investigations.