Project Report

Immunomodulatory effects of Tulsi (*Ocimum sanctum* linn.) on healthy human subjects

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Institute
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Supported by
Department of AYUSH, Ministry of Health & Family Welfare, Govt. of India, New Delhi
FINAL REPORT

1. Title of the Project: IMMUNOMODULATORY EFFECTS OF TULSI (OCIMUM SANCTUM LINN.) ON HEALTHY HUMAN SUBJECTS

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4. Other Scientific Staff engaged in the study (One post of SRF):
   Dr. B.D. BAMOLA, SRF (12/ 2007 to 01/ 2009)
   Dr. S. MONDAL, SRF (02/2009 to 11/2009)
   Dr. S. BAMOLA, SRF (12/2009 to 01/2011)

5. Non-Scientific Staff engaged in the study (One post of lab attendant):
   Mr. Kiran Pal, Lab Attendt (01/2008 to 12/2010)

6. Implementing Institution and other collaborating Institutions:
   AIIMS, New Delhi
   IIT Delhi, New Delhi
   ICMR-Institute of Pathology, New Delhi
   CCRAS, New Delhi

7. Date of commencement: 23 December 2007

8. Duration: Three Years (36 Months)

9. Date of completion: January 2011

10. Objectives as approved: Achieved
11. Deviation made from original objectives if any, while implementing the project and reasons thereof.

There were two objectives aimed to achieve in the research proposal as follows.

- *To study the chemical composition of Tulsi leaves collected from different geographical locations in India.*

- *To evaluate the effects of Tulsi, if any, on immune parameters of healthy human subjects.*

Both the objectives, as stated above, are achieved however some modifications were done keeping in mind the suggestions given by the CCRAS while granting the sanction of the project and practical problem faced during the implementation. In original plan, to achieve first objective, there was a proposal to study the chemical composition of Tulsi leaves’ essential oil and ethanolic extracts from different locations in India. While granting the sanction it was decided during PEC [Project Evaluation Committee] meeting that ethanolic extract will be supplied by the CCRAS which is to be used for the achievements of the second objective. Thus, chemical analysis of essential oil obtained from the dried leaf of Tulsi was analyzed by using Gas Chromatography/ Mass Spectrometry [GC/MS] and GC at IIT Delhi.

Regarding second objective, we conducted a double blinded randomized controlled trial in healthy volunteers. In this trial, we have evaluated Th1 and Th2 cytokines, Phenotyped T-cells [CD3+CD4+, CD3+CD8+], B-cells [CD19+] and NK-cells [CD16+ CD56+] along with clinical biochemistry parameters. CD60+ and CD69+ is expressed on the surface of the activated cells in response to challenge in the form of Lipopolysaccharide or Phytoagglutinin PHA, since we have not used Flowcytometry to determine intracellular expression of Th1 and Th2 cytokines thus these markers were not studied. Due to technical problem faced in studying cytokine using flowcytometer, we decided to use ELISA to measure the Th1 and Th2 cytokines. We could study the main cytokines belong to Th1 and Th2 family due to limitations of funds.

Number of subjects to be studied in the projected in the proposal had to be reduced because there was no release of funds for more than one a year. However, there was no compromise on the quality of trial. We have conducted clinical trial with 80% power of study and 95% CI.

12. Experimental work giving full details of experimental set up, methods adopted, data collected supported by necessary tables, charts, diagrams and photographs.
12.A. Objective 1: To study the chemical composition of Tulsi leaves collected from different geographical locations in India.

Introduction

Chemical composition of aromatic medicinal plants varies with season to season and place of cultivation. Tulsi plant grows almost in every region of India, which has varying geographical and environmental conditions in different regions. The present study was designed to compare chemical constituents of essential oils obtained from two locations namely Lucknow and Chennai for dry leaves, and from Delhi for fresh leaves. We observed that there were differences in the composition of essential oils of Tulsi obtained from leaves.

12.A. 1: Materials and methods used to achieve objective no. 1:

12.A.1. a: Collection of dried leaves of Ocimum sanctum

The dried leaves of Ocimum sanctum (Linn.) were collected and supplied by Indo-Israel Trading Corporation (IITC), Lucknow. The leaves of Tulsi were collected from southern part of India, Chennai (13°04' N, 80°17' E) and central part of India, Lucknow (26°30' to 27°10' N, 80°30' to 81°13’ E) in the month of October-November, when essential oil content of the leaves is reported to be highest (Kothari et al 2004; Gupta, 1996). Drying was done in shade and was vacuum packed in 500 g each, in order to reduce any kind of loss of essential oil due to evaporation. Locations of the sampling area have been shown in map 1.

12.A.1. b: Collection of fresh leaves of Ocimum sanctum

In order to get fresh leaves of Tulsi, seeds were sown in small plot size at micro-model herbal garden at Indian Institute of Technology Delhi, New Delhi (28° 35' N, 77° 12' E). Saplings of Tulsi were planted in the month of March. Middle aged fresh leaves of Tulsi were plucked during the month of October-November in the morning between 9-10 a.m. (IST) when dew was less and temperature was also not so high. Locations of the sampling area have been shown in map 1.

12.A.1.d: Identification of plant material

The samples of Ocimum sanctum Lin. has been identified by Dr. E Roshini Nayar, Head, Taxonomy Division, National Bureau of Plant Genetics and Research (NBPGN), New Delhi. The sample specimen has been deposited at NBPGN (No NH/NBPGN/1103/3650).
12.A.1.e: Extraction of essential oil from dried leaves of Tulsi

The essential oils of *Tulsi* were extracted by hydro-distillation using Clevenger’s type apparatus. Two hundred grams of dried leaves of *Tulsi* were put in 5 L round bottomed flask and was filled with de-ionized water up to two third marks of the flask. Electrical heating mantle was used to heat the water. The flask was fitted with Clevenger and condenser. The essential oil remained floated in the oil collection area. After 4-5 hours of heating, when no visible essential oil was seen in the condenser area, essential oil was collected after draining the bottom water layer. Sodium hypo-carbonate was used to absorb any remaining moisture content in the essential oil.

12.A.1.f: Extraction of essential oil from fresh leaves of Tulsi

Similarly, essential oil from fresh leaves of *Tulsi* was also extracted by hydro-distillation process.

12.A.1.g: Measurement of yield of essential oils of Tulsi

The yields of essential oils of *Tulsi* were measured with the help of micropipettes and silicon coated micro tips. The yield is expressed as percentages of weight / volume (w/v).

12.A.1.h: Storage of essential oil

The essential oil of *Tulsi* was stored in the refrigerator at 4°C in micro tubes for analysis.

12.A.1.i: Analysis of essential oil for comparative study

Essential oils from dried and fresh leaves of *Tulsi* were analyzed by Gas Chromatography (GC) and Gas Chromatography/ Mass Spectrometry (GC/MS).

12.A.1.i.1: Principle of GC/MS

The Gas Chromatography/Mass Spectrometry (GC/MS) instrument separates chemical mixtures (the GC component) and identifies the components at a molecular level (the MS component). The GC works on the principle that a mixture will separate into individual substances at different temperatures when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule. (http://www.cpeo.org/techtree/ttdescript/msgc.htm)
12.A.1.i.2: Gas-Chromatography (GC) analysis
For GC, Nucon 5765 Gas Chromatograph was used. Capillary column was RTX-5 MS (30 m×0.25 mm, 0.25 μm film coating). Injection temperature was 240 °C. Helium was used as the carrier gas (1.2 ml/min), 0.5µl oil was injected into the column. Component separation was achieved following a linear temperature program of 60°-210 °C (3 °C/min). Percentage composition was calculated using peak normalization method assuming equal detector response.

12.A.1.i.3: Gas Chromatography/Mass Spectrometry (GC/MS)
Chemical constituents of fresh and dried leaves essential oils of Tulsi were identified by GC/MS. The EO was analyzed by ThermoQuest Trace GC-2000 interfaced with Finnigan Mat Polaris-Q ion trap mass spectrometer, with the same column and temperature programmed as in GC.

12.A.1.i.4: MS conditions
The ion source temperature at 200 °C, ion inlet temperature at 210 °C and ionization voltage 70 eV (electron Volt), and detector voltage 1.5 Volts.

12.A.1.i.5: Identification of chemical constituents
The different components of the oil were identified by comparison of mass spectral data with those available in spectral libraries (NIST and Wiley GC-MS databases). The compound identification was finally confirmed by comparison of their relative retention indices (Van Den Dool, 1963) with literature values (Adams, 1989; Davies, 1990).

12.A.1.i.6: Relative Retention Indices (RRI) or Kovat’s Indices
According to IUPAC (1997), the Relative Retention Indices or Kovát’s (retention) index expresses the number of carbon atoms (multiplied by 100) of a hypothetical normal alkane which would have an adjusted retention time identical to that of the peak of interest when analyzed under identical conditions. When the sample is run, the retention time of the desired component is first located between a pair of retention times of two adjacent hydrocarbons in the series and RRI of the sample peak is calculated using the following equation (Rout, 2008). For example, the sample peak has appeared between the standard peaks of hydrocarbons containing N and N+1 carbon (C) atoms, then
\[ RRI \ of \ the \ sample = 100N + 100 \frac{\log RTof\ C_{N+1} - \log RTof\ sample}{\log RTof\ C_{N+1} - \log RTof\ C_N} \]
Map 1: Location of Sample collection shown by arrow heads in political map of India
(Original map downloaded from Survey of India, Govt. of India, www.surveyofindia.gov.in)

Different states and union territories of Republic of India are colored separately. Arrow heads showing the locations of Tulsi sample collection.
12.A.2: Results: Composition of essential oil of leaves of *Tulsi* from Lucknow, Chennai and Delhi

12.A.2.a: Yield of essential oil of *Tulsi* from fresh and dried leaves

It was observed that the yield of essential oils from the dried leaves of *Tulsi* from Lucknow and Chennai samples were similar i.e. 1% weight/volume (w/v). However, essential oil from fresh leaves of *Tulsi* grown at Indian Institute of Technology Delhi was only 0.4% weight/volume (Table 1). It was found that the weight of one microliter essential oil of *Tulsi* was one microgram only and this conversion has been used in the antimicrobial assay.

**Table 1:** Yield of essential oil from fresh and dried leaves of *Tulsi* from different locations.

<table>
<thead>
<tr>
<th></th>
<th>Yield (w/v) of essential oil of <em>Tulsi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh leaves from</td>
<td>Dried leaves from</td>
</tr>
<tr>
<td>Delhi samples (n=3)</td>
<td>Lucknow samples (n=3)</td>
</tr>
<tr>
<td>0.40 ± 0.05 %</td>
<td>1.00 ± 0.10 %</td>
</tr>
<tr>
<td>1.00 ± 0.09 %</td>
<td>1.00 ± 0.09 %</td>
</tr>
</tbody>
</table>

Values presented in mean±SD.
Fig 1: Representative Gas chromatogram of dried leaves essential oil of *Tulsi* from Lucknow samples

![Gas chromatogram of dried leaves essential oil of *Tulsi* from Lucknow samples](image1)

Retention time

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Fig 2: Representative Gas chromatogram of dried leaves essential oil of *Tulsi* from Chennai samples

![Gas chromatogram of dried leaves essential oil of *Tulsi* from Chennai samples](image2)

Retention time
12.A.2.b: Composition of dried leaves essential oil of Tulsi from Lucknow and Chennai

The GC/MS analysis of Lucknow samples revealed 19 compounds, amounting to 97.4±3.45 percent of total identified compounds. While the samples from Chennai, 22 compounds were identified, amounting to 97.3±3.20 percent of total identified compounds. The major constituents of essential oils of dried leaves Tulsi obtained from Lucknow and Chennai samples are shown in table 2.

Samples from Lucknow and Chennai had 18 common compounds (Table 6), though with varying percentage. The Lucknow samples had one unique compound, Germa crene D (3.3±0.11 %) which was not present in the Chennai sample while the Chennai samples had four unique compounds β-pinene (T), β-copaene (0.5±0.05%), Chamigrene-β (1.9±0.05%) and α-trans bergamotene (1.1±0.10%) amounting to a total of 3.5±0.2%, which were not found in the Lucknow samples.

Caryophyllene oxide was the single largest constituent in the samples from Lucknow and Chennai having 41.6±1.55 and 35.9±2.11 percentages respectively. Caryophyllene oxide (41.6±1.55%), caryophyllene (18.0±0.19%), elemene (10.9±0.85%) eugenol (10.7±0.25%) and germa crene-A (3.4±0.11%) were the five major elements in the Lucknow samples (Table 4) which constituted 84.6±2.95 percent of total indentified compounds. In the Chennai samples (Table 4), Caryophyllene oxide (35.9±2.11%), caryophyllene (17.9±0.05%), eugenol (11.9±0.15%), elemene (7.7±0.10%) and methyl chavicol (6.7±0.01%) were the five major elements which constituted 80.1±2.42 percent of total indentified compounds. It was observed that the constituents that were common in both of the samples constituted more than 90% of total identified compounds (Table 6). All values presented here is the mean ± SD until otherwise described.
12.A.2.c: Composition of fresh leaves essential oil of Tulsi from Delhi

Essential oil obtained from fresh leaves of Tulsi grown in Delhi had given a yield of 0.40% w/v (Table 1). Five major constituents of fresh leaves essential oil were eugenol (57.94 ±2.56%), β-caryophyllene (15.32 ±0.87%), germacrene-A (9.10 ±1.01%), β-elemene (7.57 ±1.20%) and caryophyllene oxide (3.30 ±0.60%) which constituted 93.23±6.24 percent of the total identified compounds (Table 3). β-pinene, β-copaene, β-bourbene, chamigrene-β, α-trans bergamotene and α-selinene were not presents in the fresh leaves essential oil obtained from Delhi samples. There was no unique compound in Delhi samples, however, all the constituents present in the Delhi samples were common to Lucknow samples but with a varying percentages (Table 6). Eugenol (57.94 ±2.56%) was the major constituent in the fresh leaves essential oil which constituted more than half of the total identified compounds. Table 3 shows the chemical compounds identified in fresh leaves of Tulsi from Delhi.
Table 2: Major chemical constituents of essential oils of dried leaves of *Tulsi* from Lucknow (Uttar Pradesh) and Chennai (Tamilnadu)

*RRI= Relative Retention Index, KI= Kovat’s Index

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Lucknow (Uttar Pradesh) (% Mean ± SD)</th>
<th>Chennai (Tamilnadu) (% Mean ± SD)</th>
<th>RRI/KI* Calculated</th>
<th>RRI / KI Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-pinene</td>
<td>T (Trace, &lt;0.1%)</td>
<td>0.5±0.05</td>
<td>932</td>
<td>932</td>
</tr>
<tr>
<td>Camphene</td>
<td>T</td>
<td>T</td>
<td>949</td>
<td>951</td>
</tr>
<tr>
<td>β-pinene</td>
<td>__</td>
<td>T</td>
<td>978</td>
<td>980</td>
</tr>
<tr>
<td>Limolene</td>
<td>T</td>
<td>0.1±0.05</td>
<td>1029</td>
<td>1031</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>T</td>
<td>T</td>
<td>1036</td>
<td>1033</td>
</tr>
<tr>
<td>Linalool</td>
<td>T</td>
<td>3.3±0.05</td>
<td>1091</td>
<td>1098</td>
</tr>
<tr>
<td>Bornol</td>
<td>0.5±0.05</td>
<td>0.4±0.05</td>
<td>1167</td>
<td>1165</td>
</tr>
<tr>
<td>Methyl chavicol</td>
<td>0.1</td>
<td>6.7±0.05</td>
<td>1191</td>
<td>1195</td>
</tr>
<tr>
<td>α-Cubebene</td>
<td>0.3±0.05</td>
<td>0.2±0.05</td>
<td>1347</td>
<td>1351</td>
</tr>
<tr>
<td>Eugenol</td>
<td>10.7±0.25</td>
<td>11.9±0.15</td>
<td>1359</td>
<td>1356</td>
</tr>
<tr>
<td>α-Copaene</td>
<td>__</td>
<td>0.5±0.05</td>
<td>1364</td>
<td>1378</td>
</tr>
<tr>
<td>β-Bourbonene</td>
<td>0.6±0.05</td>
<td>T</td>
<td>1384</td>
<td>1384</td>
</tr>
<tr>
<td>β-Elemene</td>
<td>10.9±0.85</td>
<td>7.7±0.1</td>
<td>1395</td>
<td>1391</td>
</tr>
<tr>
<td>E-Caryophyllene</td>
<td>18.0±0.2</td>
<td>17.9±0.05</td>
<td>1419</td>
<td>1418</td>
</tr>
<tr>
<td>α-E-Bergamotene</td>
<td>__</td>
<td>1.1±0.1</td>
<td>1434</td>
<td>1436</td>
</tr>
<tr>
<td>α-Humulene</td>
<td>1.3±0.15</td>
<td>0.9±0.05</td>
<td>1449</td>
<td>1454</td>
</tr>
<tr>
<td>β-Chamigrene</td>
<td>__</td>
<td>1.9±0.05</td>
<td>1472</td>
<td>1475</td>
</tr>
<tr>
<td>Germacrene-D</td>
<td>3.3±0.1</td>
<td>__</td>
<td>1479</td>
<td>1480</td>
</tr>
<tr>
<td>β-Selinene</td>
<td>2.7±0.05</td>
<td>1.9±0.05</td>
<td>1486</td>
<td>1485</td>
</tr>
<tr>
<td>α-Selinene</td>
<td>3.3±0.05</td>
<td>5.7±0.05</td>
<td>1497</td>
<td>1494</td>
</tr>
<tr>
<td>Germacrene A</td>
<td>3.4±0.1</td>
<td>0.2±0.05</td>
<td>1506</td>
<td>1503</td>
</tr>
<tr>
<td>δ-Cadinene</td>
<td>0.70±0.05</td>
<td>0.5±0.1</td>
<td>1525</td>
<td>1524</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>41.6±1.5</td>
<td>35.9±2.1</td>
<td>1587</td>
<td>1585</td>
</tr>
<tr>
<td>Total identified</td>
<td><strong>97.4±3.45</strong></td>
<td><strong>97.3±3.20</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3 Major chemical constituents of essential oils of fresh leaves of *Tulsi* from Delhi samples.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Delhi Fresh leaf EO (%)</th>
<th>RRI Calculated</th>
<th>RRI / KI literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-pinene T (Trace, &lt;0.1%)</td>
<td>932</td>
<td>939</td>
<td></td>
</tr>
<tr>
<td>Camphene T</td>
<td>949</td>
<td>951</td>
<td></td>
</tr>
<tr>
<td>Limonene T</td>
<td>1027</td>
<td>1031</td>
<td></td>
</tr>
<tr>
<td>1,8-cineole 0.9±0.05</td>
<td>1036</td>
<td>1033</td>
<td></td>
</tr>
<tr>
<td>Linalool T</td>
<td>1091</td>
<td>1098</td>
<td></td>
</tr>
<tr>
<td>Borneol 0.15±0.1</td>
<td>1167</td>
<td>1165</td>
<td></td>
</tr>
<tr>
<td>Methyl chavicol 0.1±0.05</td>
<td>1191</td>
<td>1195</td>
<td></td>
</tr>
<tr>
<td>α-cubebene 0.1±0.05</td>
<td>1347</td>
<td>1351</td>
<td></td>
</tr>
<tr>
<td>Eugenol 57.9±2.6</td>
<td>1359</td>
<td>1356</td>
<td></td>
</tr>
<tr>
<td>β-elemene 7.6±0.9</td>
<td>1395</td>
<td>1391</td>
<td></td>
</tr>
<tr>
<td>E-caryophyllene 15.3±1.0</td>
<td>1419</td>
<td>1418</td>
<td></td>
</tr>
<tr>
<td>α-humulene 0.9±0.05</td>
<td>1449</td>
<td>1454</td>
<td></td>
</tr>
<tr>
<td>Germacrene D 0.15±0.05</td>
<td>1479</td>
<td>1480</td>
<td></td>
</tr>
<tr>
<td>β-Selinene 0.5±0.05</td>
<td>1486</td>
<td>1485</td>
<td></td>
</tr>
<tr>
<td>Germacrene A 9.1±1.2</td>
<td>1506</td>
<td>1503</td>
<td></td>
</tr>
<tr>
<td>δ-cadinene 0.15±0.05</td>
<td>1525</td>
<td>1524</td>
<td></td>
</tr>
<tr>
<td>Caryophyllene oxide 3.3±0.05</td>
<td>1587</td>
<td>1585</td>
<td></td>
</tr>
<tr>
<td><strong>Total identified</strong></td>
<td><strong>96.15±6.20</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*RRI= Relative Retention Index, KI= Kovat’s Index*
Table 4: Five major compounds in dried leaves essential oil of *Tulsi* from Lucknow and Lucknow samples.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Lucknow (Uttar Pradesh) (%)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eugenol</td>
<td>10.7±0.25</td>
<td></td>
</tr>
<tr>
<td>β-Elemene</td>
<td>10.9±0.85</td>
<td></td>
</tr>
<tr>
<td>E-Caryophyllene</td>
<td>18.0±0.2</td>
<td></td>
</tr>
<tr>
<td>Germacrene A</td>
<td>3.4±0.1</td>
<td></td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>41.6±1.5</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>84.6±2.90</strong></td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Five major compounds of fresh leaves essential oil of *Tulsi* from Delhi samples.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Delhi Fresh leaf EO (%)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eugenol</td>
<td>57.9±2.6</td>
<td></td>
</tr>
<tr>
<td>β-elemene</td>
<td>7.6±0.9</td>
<td></td>
</tr>
<tr>
<td>E-caryophyllene</td>
<td>15.3±1.0</td>
<td></td>
</tr>
<tr>
<td>Germacrene A</td>
<td>9.1±1.2</td>
<td></td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>3.3±0.05</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>93.2±5.75</strong></td>
<td></td>
</tr>
</tbody>
</table>
Table 6: Common compounds in essential oils of dried leaves of *Tulsi* obtained from Lucknow and Chennai and fresh leaves essential oil obtained from Delhi samples.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Lucknow (Uttar Pradesh) (%, Mean ± SD)</th>
<th>Chennai (Tamilnadu) (%, Mean ± SD)</th>
<th>Delhi Fresh leaf EO (%, Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-pinene</td>
<td>T (Trace, &lt;0.1%)</td>
<td>0.5±0.05</td>
<td>T</td>
</tr>
<tr>
<td>Camphene</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>Limolene</td>
<td>T</td>
<td>0.1±0.05</td>
<td>T</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>T</td>
<td>T</td>
<td>0.9±0.05</td>
</tr>
<tr>
<td>Linalool</td>
<td>T</td>
<td>3.3±0.05</td>
<td>T</td>
</tr>
<tr>
<td>Borneol</td>
<td>0.5±0.05</td>
<td>0.4±0.05</td>
<td>0.15±0.1</td>
</tr>
<tr>
<td>Methyl chavicol</td>
<td>0.1±0.01</td>
<td>6.7±0.05</td>
<td>0.1±0.05</td>
</tr>
<tr>
<td>α-Cubebene</td>
<td>0.3±0.05</td>
<td>0.2±0.05</td>
<td>0.1±0.05</td>
</tr>
<tr>
<td>Eugenol</td>
<td>10.7±0.25</td>
<td>11.9±0.15</td>
<td>57.9±2.6</td>
</tr>
<tr>
<td>β-Bourbonene</td>
<td>0.6±0.05</td>
<td>T</td>
<td>-</td>
</tr>
<tr>
<td>β-Elemene</td>
<td>10.9±0.85</td>
<td>7.7±0.1</td>
<td>7.6±0.9</td>
</tr>
<tr>
<td>E-Caryophyllene</td>
<td>18.0±0.2</td>
<td>17.9±0.05</td>
<td>15.3±1.0</td>
</tr>
<tr>
<td>α-Humulene</td>
<td>1.3±0.15</td>
<td>0.9±0.05</td>
<td>0.9±0.05</td>
</tr>
<tr>
<td>β-Selinene</td>
<td>2.7±0.05</td>
<td>1.9±0.05</td>
<td>0.5±0.05</td>
</tr>
<tr>
<td>α-Selinene</td>
<td>3.3±0.05</td>
<td>5.7±0.05</td>
<td>-</td>
</tr>
<tr>
<td>Germacrene A</td>
<td>3.4±0.1</td>
<td>0.2±0.05</td>
<td>9.1±1.2</td>
</tr>
<tr>
<td>δ-Cadinene</td>
<td>0.70±0.05</td>
<td>0.5±0.1</td>
<td>0.15±0.05</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>41.6±1.5</td>
<td>35.9±2.1</td>
<td>3.3±0.05</td>
</tr>
<tr>
<td><strong>Total identified</strong></td>
<td><strong>94.1±3.36</strong></td>
<td><strong>93.8±3.00</strong></td>
<td><strong>96.0±6.15</strong></td>
</tr>
</tbody>
</table>
12.B: Objective No.2: To evaluate the effects of Tulsi, if any, on immune parameters of healthy human subjects.

12.B.1: Introduction

*Tulsi* or Holy Basil (*Ocimum sanctum* Linn) is widely distributed in India from the sea level to up to 1800 meters altitude in the Himalayas (Wealth of India, 1991). Its medicinal properties have been described in the Indian medicinal text *Ayurveda* (The science of Life) which is believed to be about 5000 years old. Traditionally, various parts of this plant have been used for different ailments such as cough and cold, asthma, bronchitis, digestive disorders, skin problems, eye and ear infections, undifferentiated fever, snake and scorpion bites (Ghosh, 1995). Scientific explorations of traditional medicinal claims of *Tulsi* got the momentum in the middle part of the 20th century. Most of the scientific evidences of medicinal properties of this plant were observed largely on experimental animal studies with only a few human studies. These studies have shown notable properties such as antimicrobial, adaptogenic, anti-diabetic, hepato-protective, anti-inflammatory, anti-carcinogenic, radio-protective, neuro-protective, cardio-protective and larvicidal / mosquito repellent of different parts of the *Tulsi* plant (Mondal et al, 2009). Experimental animal studies have clearly shown immunomodulatory properties in the extract of *Tulsi* leaves (Godhwani et al, 1988; Singh et al, 1995; Mediratta et al, 2002; Mukherjee et al, 2005). Immune system of human is very complex and there lies a delicate balance between health and disease. Any substance, synthetic or biological, which can enhance, suppress or modulate the immune system, is called an immunomodulator (Agarwal and Singh, 1991). It is often believed in India that taking *Tulsi* leaves on empty stomach is beneficial and improves immunity, thus the present study was designed to determine the immunomodulatory properties of *Tulsi* leaf ethanolic (70%) extract in healthy volunteers through a double-blind randomized controlled trial.
12.B.2. Material and methods

12.B.2.1 Study design

A double-blind randomized controlled trial in a cross-over format with a washout period was designed to study the immunomodulatory effects of ethanolic extract of *Tulsi* leaf in healthy volunteers. The study was approved by the institutional ethics committee on research involving human subjects and registered with clinical trial registry of India (No. CTRI/2009/091/000350).

**Fig : Experimental Design**

12.B.2.2 Recruitment of volunteers

Twenty four healthy volunteers were enrolled in the study after initial screening of 45 subjects. Following were the inclusion criteria for the enrollment of healthy volunteers i.e. a) age 18-60 years, either sex, b) devoid of any medication during last one month. Subjects suffering from different diseases/disorders and/or having any kind of allergy, undergone surgery during last one year, received organ transplant, chronic smokers, underlying conditions which might affect immunity and pregnant/lactating women were excluded from the study. Enrolled individuals were randomized into two groups. Subjects and the staff directly involved with the study subjects and data analysis were blinded about the interventional capsules. Allocation of *Tulsi* extract or placebo was concealed in opaque envelop.

12.B.2.3 Intervention

Interventional drug (70% ethanolic extract of *Ocimum sanctum* Linn. leaves, ‘*Tulsi* extract’) was supplied by the Central Council for Research in Ayurveda and Siddha, Department of AYUSH, Ministry of Family Health and Welfare, Government of India. Placebo that contained sucrose was supplied by the Dabur Pharmaceutical (India) Ltd, Ghaziabad (U.P.), India. Three hundred milligram capsules of *Tulsi* extract or placebo (sucrose) were prepared by the Dabur
Pharmaceutical (India) Ltd. To avoid identification, the shape, size, color and packaging of both, *Tulsi* extract and placebo, were similar. Once the volunteer met the inclusion criteria, a written informed consent was obtained and the intervention was allocated as per the randomized sequence. The capsules were administered on empty stomach for four weeks followed by a washout period of three weeks before the volunteer cross-over to the next intervention.

### 12.B.2.4 Blood sampling

Venous blood samples were collected through *vene puncture* of antecubital vein at four different time points i.e. i) at baseline, ii) after four weeks of placebo or *Tulsi* extract, iii) after washout period of three weeks and lastly, iv) after completion of four weeks in crossover intervention period. The compliance of the capsule intake was monitored by reminding the volunteers personally or telephonically twice a week and also from the numbers of unused capsules returned by the subjects. Two (n=2) volunteers were lost during follow-up.

### 12.B.4.5 Biochemical parameters

As side effects of any drug intervention is largely reflected by abnormality in the biochemical tests. In this study, biochemical parameters were evaluated to monitor any adverse effect that might arise due to intervention. Semi automated clinical biochemistry analyzer (Labmate 20, Trivitron, Bangalore, India) and commercial kits (Transasia, India; Randox, United Kingdom) were used in this study.

### 12.B.2.6 Whole blood culture and cytokine assay

Mitogen induced secretion of Th1 and Th2 cytokines [Interferon-gamma (INF-γ) and Interleukin-4 (IL-4)] levels in whole blood culture were monitored at four different time points using ELISA. Secreted levels of cytokines (IFN-γ and IL-4) were studied as per Villard et al, 1999, in the whole blood culture. Briefly, venous blood obtained from volunteers was cultured in RPMI-1640 medium and was stimulated with *Escherichia coli* derived lipopolysaccharide (LPS) at a final concentration of 25 μg/ml and phytohaemagglutinin (PHA) at a final concentration of 5 μg/ml (Calbiochem, Germany). Culture supernatant were harvested after 24 hours of incubation at 37°C in 5% CO₂ humidified chamber and cytokine levels were measured using commercial ELISA kits (Thermo Scientific, Illinois, USA) with a sensitivity of less than 2pg/ml. Samples for ELISA test was performed as per the manufacturer’s instructions and reading was taken using
Bio-Rad ELISA reader (Benchmark Plus, CA, USA) and Microplate manager software version 5.2.1.

12.B.2.7 Flowcytometry for phenotyping of lymphocytes

Phenotyping of T-cells (CD3⁺CD4⁺, CD3⁺CD8⁺), B-cells (CD19⁺) and NK-cells (CD16⁺CD56⁺) was carried out using Flowcytometer (FACSCaliber, Beckton Dikinson) and CELLQuest® software. CD3 labeled with Phycoerythrin Cyanin 5 (PE Cy 5), CD4 labeled with Phycoerythrin (PE), CD8 labeled with Fluorescein isothiocyanate (FITC), CD19 labeled with FITC, CD16 labeled with FITC and CD56 labeled with PE were procured commercially (BD-Pharmigen, USA) and the samples were processed as per the manufacture’s instructions.

12.B.2.7 Statistical analysis

Two-way Analysis of Variance (ANOVA) for crossover design was used to test the statistical significance of the results. Independent t-test was applied to compare the baseline of two groups. The results were considered significant if the p-value was ≤ 0.05 for period effects and
intervention effects. However, p-value of ≤0.10 for carryover effects was considered significant (Jones and Kenward, 2003). All statistical analysis was carried out using Stata 9.0 software (Statacorp, Texas, USA). The blinding was decoded only after the statistical analysis.

12.B.3. Results

12.B 3.1 Study population, compliance and carry over effects

All baseline parameters of both placebo and Tulsi extract groups were comparable and no significant differences were noted (Table 7). No significant adverse effects of the intervention were noted amongst study individuals during the study period of 11 week except in two subjects, one of the subjects complained of nausea while the other had loose motions, after first visit to the laboratory. These two subjects could not complete the study and their data were excluded from the analysis (Figure 4). Further, there were no carry over or sequence of effect (i.e. whether the placebo or Tulsi extract administered initially or later) of the placebo or Tulsi extract intervention. Thus, the data of the subjects who received Tulsi extract intervention either before cross-over or after cross-over were grouped together and similarly placebo treated subjects were also grouped together for analysis and interpretation. The compliance of the capsule intake was very satisfactory and compliance rate was more than 95 %.
12.B 3.2 Effects of Tulsi extract on clinical biochemistry parameters of healthy volunteers.

In order to monitor possible side effects of Tulsi extract administration in healthy volunteers, their clinical biochemistry parameters were monitored. It was observed that intake of Tulsi extract did not affect clinical biochemistry parameters in a harmful manner. In a positive note it was observed that the subjects who had an elevated levels of total cholesterol and triglycerides at baseline showed a significant reduction in total cholesterol and a reduction trend in triglycerides thus it was found to have cardioprotective properties. [Results are published, Mondal et al, 2012, Journal of Preventive cardiology] [Table 8 and 9]
Figure 4: Flowchart of recruitment of healthy volunteers
Table 7: Comparison of basic parameters of volunteers at the baseline (0 weeks).

<table>
<thead>
<tr>
<th>Basic parameters</th>
<th>Baseline Placebo-\textit{Tulsi} sequence (n=12)</th>
<th>Baseline \textit{Tulsi}- Placebo sequence (n=10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27.5±4.75</td>
<td>26.5±3.43</td>
<td>0.585</td>
</tr>
<tr>
<td>Height</td>
<td>166.16±7.62</td>
<td>166.1±5.40</td>
<td>0.981</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.5 ±10.52</td>
<td>65.8±6.05</td>
<td>0.391</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>22.36±2.52</td>
<td>23.80±1.13</td>
<td>0.111</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>109.16±7.50</td>
<td>113.2±7.37</td>
<td>0.220</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>74.16±4.62</td>
<td>76.2±3.45</td>
<td>0.265</td>
</tr>
</tbody>
</table>

All values presented in mean±SD. p-Value ≤0.05 was considered as significant.

Independent t-test applied.
Table 8: Intervention effects of *Tulsi* extract or placebo (sucrose) capsules on biochemical parameters after 4 weeks.

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Sequence of interventions</th>
<th>Baseline (at 0 week)</th>
<th>4 weeks after first intervention (4th week)</th>
<th>4 weeks after second intervention (11th Week)</th>
<th>Baseline comparison p-Value#</th>
<th>Period effects p-Value a</th>
<th>Carryover effects p-Value b</th>
<th>Intervention effects p-Value^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (fasting) (mg/dl)</td>
<td>P</td>
<td>99.25±3.84</td>
<td>99.58±4.39</td>
<td>96.33±3.60</td>
<td>0.546</td>
<td>0.109</td>
<td>0.940</td>
<td><strong>0.057</strong></td>
</tr>
<tr>
<td></td>
<td>TE (n=10)</td>
<td>98±3.85</td>
<td>97.7±3.83</td>
<td>99±3.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>P</td>
<td>187±48.04</td>
<td>188.91±44.97</td>
<td>174±16.49</td>
<td>0.232</td>
<td>0.123</td>
<td>0.827</td>
<td><strong>0.003</strong></td>
</tr>
<tr>
<td></td>
<td>TE (n=10)</td>
<td>206.18±42.76</td>
<td>165.25±12.92</td>
<td>200.6±42.76</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Protein (g/ml)</td>
<td>P</td>
<td>7.34±0.76</td>
<td>7.59±0.63</td>
<td>7.27±0.79</td>
<td>0.515</td>
<td>0.711</td>
<td>0.608</td>
<td>0.838</td>
</tr>
<tr>
<td></td>
<td>TE (n=10)</td>
<td>7.16±0.54</td>
<td>7±0.52</td>
<td>7.16±0.54</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Urea Nitrogen (mg/dl)</td>
<td>P</td>
<td>22±5.93</td>
<td>22.25±5.70</td>
<td>21.9±5.5</td>
<td>0.365</td>
<td>0.406</td>
<td>0.555</td>
<td>0.511</td>
</tr>
<tr>
<td></td>
<td>TE (n=10)</td>
<td>19.08±2.48</td>
<td>19.8±2.48</td>
<td>21.9±7.60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>P</td>
<td>0.84±0.25</td>
<td>0.82±0.22</td>
<td>0.83±0.21</td>
<td>0.780</td>
<td>0.139</td>
<td>0.819</td>
<td>0.766</td>
</tr>
<tr>
<td></td>
<td>TE (n=10)</td>
<td>0.85±0.07</td>
<td>0.83±0.12</td>
<td>0.85±0.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values presented in mean±SD. P- Placebo, TE- *Tulsi* extract, # independent t-tests used. ^abc Two-way ANOVA for crossover design. ±^abc p-Value ≤0.05 and ^b p-Value ≤0.10 were considered as significant. * Significant decrease.
12.B 3.2 Effects on Th1 & Th2 cytokine release

In *in-vitro* culture of whole blood stimulated with PHA and LPS, there were no significant differences in the IFN-γ (Th1) and IL-4 (Th2) cytokines at the baseline of both the groups. However, it observed that there were significant increase in the levels of both IFN-γ and IL-4 (*p*=0.039 and *p*=0.001, respectively) in the blood samples of *Tulsi* extract intervention group. This increase did not continue when subjects were crossed-over to placebo intervention after the wash out period. (Figure 5 & 6)

**Table 9:** Effects of *Tulsi* extract or placebo (sucrose) capsules on Triglycerides after 4 weeks of intervention.

<table>
<thead>
<tr>
<th></th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td><strong>Placebo (n=12)</strong></td>
<td>57.5 (25-194)</td>
</tr>
<tr>
<td><strong>Tulsi extract (10)</strong></td>
<td>78.5 (25-350)</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.128</td>
</tr>
</tbody>
</table>

Value presented in median (range). *p*-Value ≤0.05 was considered as significant. Wilcoxon singed-rank test applied.
Table 10: Intervention effects of *Tulsi* extract or placebo (sucrose) capsules on T-Lymphocytes, B-Lymphocytes and NK-cells after 4 weeks.

<table>
<thead>
<tr>
<th>Immunological parameters</th>
<th>Sequence of interventions</th>
<th>Baseline (at 0 week)</th>
<th>4 weeks after first intervention (4th week)</th>
<th>4 weeks after second intervention (11th Week)</th>
<th>Baseline comparison p-Value#</th>
<th>Period effect p-Valuea</th>
<th>Carryover effect p-Valueb</th>
<th>Intervention effect p-Valuec</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-helper cells (CD3+CD4+)</td>
<td>P (n= 12) TE</td>
<td>43.93±5.68</td>
<td>43.71±5.03</td>
<td>45.75±5.76</td>
<td>0.754</td>
<td>0.802</td>
<td>0.552</td>
<td>0.001 *</td>
</tr>
<tr>
<td></td>
<td>TE (n= 10) P</td>
<td>43.00±5.82</td>
<td>44.44±5.56</td>
<td>42.00±5.329</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-cytotoxic cells (CD3+CD8+)</td>
<td>P TE</td>
<td>29.40±4.46</td>
<td>29.59±4.65</td>
<td>29.40±4.46</td>
<td>0.520</td>
<td>0.114</td>
<td>0.256</td>
<td>0.090</td>
</tr>
<tr>
<td></td>
<td>TE P</td>
<td>27.94±4.94</td>
<td>28.05±4.82</td>
<td>26.41±4.57</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-cells (CD19+)</td>
<td>P TE</td>
<td>4.91±2.03</td>
<td>4.77±2.05</td>
<td>4.88±2.04</td>
<td>0.920</td>
<td>0.983</td>
<td>0.709</td>
<td>0.718</td>
</tr>
<tr>
<td></td>
<td>TE P</td>
<td>4.80±2.05</td>
<td>4.59±2.00</td>
<td>4.47 ±1.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK-cells (CD16+CD56+)</td>
<td>P TE</td>
<td>6.47±2.69</td>
<td>6.49±2.67</td>
<td>6.82±2.54</td>
<td>0.888</td>
<td>0.419</td>
<td>0.959</td>
<td>0.017 *</td>
</tr>
<tr>
<td></td>
<td>TE P</td>
<td>6.67±2.45</td>
<td>6.68±2.49</td>
<td>6.51±2.54</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values presented in mean±SD. P- Placebo, TE- *Tulsi* extract. # Indepident t-tests used. abc Two-way ANOVA for crossover design. a=p-Value ≤0. 05 and b=p-Value ≤0. 10 were considered as significant. *Significant increase.
Table 11: Effects of *Tulsi* extract and placebo (sucrose) on Th1 and Th2 cytokines after 4 weeks intervention.

<table>
<thead>
<tr>
<th>Th1 and Th2 Cytokines</th>
<th>Sequence of interventions</th>
<th>Baseline (at 0 week)</th>
<th>4 weeks after first intervention (4th week)</th>
<th>4 weeks after second intervention (11th week)</th>
<th>Baseline comparison p-Value</th>
<th>Period effect p-Value</th>
<th>Carryover effect p-Value</th>
<th>Intervention effect p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IFN-γ (Th1)</strong></td>
<td>P (n=12)</td>
<td>85.73±36.98</td>
<td>83.06±36.68 (4th week)</td>
<td>115.35±40.31 (11th week)</td>
<td>0.865</td>
<td>0.473</td>
<td>0.419</td>
<td><strong>0.039</strong>*</td>
</tr>
<tr>
<td></td>
<td>TE</td>
<td>88.40±38.95</td>
<td>116.95±29.00 (at 0 week)</td>
<td>79.16±40.79 (4th week)</td>
<td><strong>0.993</strong></td>
<td>0.73</td>
<td>0.346</td>
<td><strong>0.001</strong>*</td>
</tr>
<tr>
<td><strong>IL-4 (Th2)</strong></td>
<td>P (n=10)</td>
<td>9.89±4.66</td>
<td>10.74±4.78 (4th week)</td>
<td>12.97±4.65 (11th week)</td>
<td><strong>0.993</strong></td>
<td>0.73</td>
<td>0.346</td>
<td><strong>0.001</strong>*</td>
</tr>
<tr>
<td></td>
<td>TE</td>
<td>9.91±4.51</td>
<td>11.34±3.79 (at 0 week)</td>
<td>8.85±3.88 (4th week)</td>
<td><strong>0.993</strong></td>
<td>0.73</td>
<td>0.346</td>
<td><strong>0.001</strong>*</td>
</tr>
</tbody>
</table>

All values presented in mean±SD. P- Placebo, TE- *Tulsi* extract. # Indepentent t-tests used. Two-way ANOVA for crossover design. \( ^{a,b,c} \) p-Value ≤0. 05 and \(^{c} \) p-Value ≤0. 10 were considered as significant.* Significant increase.
Figure 5: Effects of placebo (sucrose) capsules on IFN-\(\gamma\) (Th1) and IL-4 (Th2) cytokines after 4 weeks of intervention (n=22)

All values in mean±SD. There was no significant difference after 4 weeks of placebo intervention. Two-way ANOVA for cross-over design applied.
Figure 6: Effects of *Tulsi* extract on Th1 and Th2 cytokines after 4 weeks of intervention (n=22).

![Graph showing effects of Tulsi extract on Th1 and Th2 cytokines](image)

All values in mean±SD. There was a significant increase in IFN-γ (Th1) (**p=0.039) and IL-4 (Th2) (*p=0.001) levels in culture supernatant of whole blood of *Tulsi* extract group after 4 weeks of intervention. Two-way ANOVA for cross-over design applied

12.B 3.3 Effects on lymphocytes

In the present study, we did not find any significant differences in the percentage of lymphocytes and NK-cell at the baseline examination in both the groups. There were also no significant differences observed in lymphocyte and NK-cell percentages in placebo group (Figure 7). However, a significant increase in the T-helper cells (p=0.001) in *Tulsi* extract intervention group (Figure 8) was observed. Apparently healthy volunteers participated in this study did not show any significant difference in the percentages of T-cytotoxic and B-cells even after 4 weeks of intervention in both the groups (Figure 7 & 8). Significant increase in the NK-cells (p=0.017) was noticed in the *Tulsi* extract group after 4 weeks of intervention (Figure 8).
Graph 1 A & B: Representative graphs of Flowcytometry.

(A) SSC vs. FSC of granulocytes, lymphocytes and monocytes.

(B) Gating of T-lymphocytes using CD3 PerCP Cy5.5 tagged Antibodies.

(SSC- H, Side Scatters- Height, FSC-H, Forward scatters-Height)
Graph 2 A, B, C: Representative graphs of T-lymphocytes.

A: CD4+ (PE tagged) and CD8+ (FITC tagged) populations in T-lymphocytes.

B: CD3+ (Per CP Cy 5.5 tagged) and CD4+ (PE, tagged) populations

C: CD3+ (Per CP Cy 5.5 tagged) and CD8+ (FITC, tagged) populations
**Figure 7:** Intervention effects of placebo (sucrose) capsules on T-Lymphocytes, B-Lymphocytes and NK-cells after 4 weeks (n=22).

All values in mean±SD. There was no significant difference after 4 weeks of placebo intervention. Two-way ANOVA for cross-over design applied.
Figure 8: Intervention effects of *Tulsi* extract capsules on T-Lymphocytes, B-Lymphocytes and NK-cells after 4 weeks (n=22).

All values in mean±SD. There was a significant increase in T-helper cells (CD3⁺CD4⁺) (**p=0.001) and NK-Cells (CD16⁺CD56⁺) (*p=0.017) after 4 weeks of *Tulsi* extract intervention. Two-way ANOVA for crossover design applied.

12 Detailed analysis of results indicating contributions made towards increasing the state of knowledge in the subject.

Details of results of the two objectives of the project have been provided in the previous section 12.A.2 and 12.B.3.

This study was first of its kind on Tulsi [*Ocimum sanctum* Linn.] to compare the essential oil of samples taken from three different locations of India, namely, Delhi, Lucknow and Chennai. The essential oil was analyzed using GC/MS [Gas chromatography/ Mass Chromatography] and found that there are common compounds with variable percentages but they also possess some specific compounds which are unique to particular location’s sample.

Double blinded randomized controlled trial [RCT] on healthy volunteers was first of its kind was designed to validate the traditional claim that taking Tulsi leaves improves immunity. There are several products of Tulsi in the market to claim it have immunostimulants but there are not a single RCT to prove it.
Thus this study is a milestone in research of medicinal properties of well known sacred plant Tulsi \textit{[Ocimum sanctum] Linn.].

13. Conclusions summarizing the achievements and indication of scope for future work.

In this study, we have found that there is a difference in the chemical composition of essential oil obtained from different locations of India. The essential oil of fresh leaves have the antimicrobial activities while dried leaves essential oil have shown the antifungal activities when used in clinical isolates of selected enteric bacteria and yeasts (Mondal et al, 2007).

When ethanolic extract (70\%) of dried Tulsi leaves where administered (300 mg/day on empty stomach) for 28 days, it increased the CD4+ (T-helper cells) and CD16+CD56+ (NK-cells) counts in the study population. These cells increase the general preparedness of immune system of the body. It can be extrapolated from the current study that in case of future immune challenge, immunity would be more efficient with the increased T-helper and NK-cells.

In vitro increase of Th1 and Th2 cytokine is very significant because Th1 cytokine helps to eliminate the intracellular infections while Th2 cytokines are helpful in mounting an effective immune challenge against extra cellular infections. These in vitro increases were also supported by the in vivo increase in the T-helper and NK cells.

To monitor any possible side effects, we monitored the physiological and biochemical parameters also. It was found that Tulsi extract has not shown any side effect in terms of biochemical parameters related to liver, kidney and heart.

Based on the current encouraging results we can envisage that, Tulsi extract might be tested for the conditions where T-helper and NK cells gets depleted or imbalance in the Th1 and Th2 cytokine secretion due to any disease condition such, HIV, Tuberculosis or Psoriasis.
14. Procurement/usage of Equipment

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of Equipment</th>
<th>Make/Model</th>
<th>Cost FE/Rs</th>
<th>Date of Installation</th>
<th>Utilization rate %</th>
<th>Remarks regarding maintenance/break down</th>
</tr>
</thead>
<tbody>
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<td>Refrigerator</td>
<td>Samsung</td>
<td>20000=00</td>
<td>16 Dec 2007</td>
<td>100%</td>
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<tr>
<td>2.</td>
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<td>20 Dec 2007</td>
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<tr>
<td>3.</td>
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<td>342500=00</td>
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<td>APC</td>
<td>2450=00</td>
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</tr>
</tbody>
</table>

15. Manuscript for Publication (300 words for possible publication in Council’s Bulletin).

Tulsi [Ocimum sanctum Linn.] is sacred plant of Indian subcontinent and several medicinal properties attributed to this plant which ranging from common cough and cold to complicated diseases like tuberculosis. Since India has a vast climatic conditions ranging from coastal areas in the south to the Himalayas in the north, serene outer Himalayas in the east and extreme desert in the west. Tulsi is used as medicinal plants and plants grown at one climatic condition might be different chemically from the other areas. Traditionally it is believed that taking Tulsi leaves on empty stomach enhances immunity.

In this project we collected samples from three geographically different locations of India and fount that there are some common compounds, with varied percentages, present in all samples but they have some unique compounds also to a particular location.

Immunomodulatory potential of this plant was studied in the healthy volunteers through a double blinded randomized controlled trial using 70% ethanolic extract of dried leaves of Tulsi [Tulsi extract]. Three hundred milligram of Tulsi extract or placebo [sucrose] was administered on empty stomach for four weeks and they were cross over to other group following three weeks wash out period and continued the capsule intake for another four weeks. Primary objective of this trial was to see the change in the Th1 and Th2 cytokines following in-vitro stimulation of blood, taken from the volunteers before and after the capsule intake, using bacterial lipopolysaccharide and phytoagglutinin. Secondary objective was to study the possible change in
the lymphocyte populations using Flowcytometry. Tertiary objective was to study any possible side effects by monitoring clinical biochemistry parameters. Independent t-test was applied to compare the baseline of placebo and treatment group. Two way ANOVA was applied to analyze the data obtained before and after the administration of Tulsi extract or placebo capsule. The results indicate that there was a significant increase in the Th1 and Th2 cytokines after 4 of Tulsi extract administration. This was further supported by the significant increase in T-helper cells and NK-cells. There were no side effects of Tulsi extract administration as indicated by clinical biochemical parameters.

16: Publications from the project [Full papers attached after page no. 55 onwards]


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