INTRODUCTION:
Ocimum sanctum L. (Tulsi) has been used for thousands of years in Ayurveda for its diverse healing properties. The effect of ocimum sanctum against diabetese mellitus as hypoglycemic effect was reported by Lokhande and Khogare, 2011. Mohamed et al., 1999 reported the effect of ocimum sanctum and Azadiracta indica on the formulation of antidiandruff herbal shampoo powder. Ocimum sanctum can be used for the treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, insect bite etc. (Prakash and Gupta, 2005). Tulsi belongs to plant family Lamiaceae. The genus Ocimum, a member of the Lamiaceae family contains 200 spp of herbs and shrubs (Simon Je et al., 1999), a source of aroma compounds andessential oils containing biologically active constituents that possess insecticidal and nematicidal properties (Deshpande RS et al., 1997; Chaterje A et al., 1997). In Ayurveda OS L. (Tulsi) has been well documented for its therapeutical potentials and described as antiasthmatic (Dashemani Shwasaharni) and cough suppressant drugs (Kaphagyna) (Khanna N et al.,2003). In traditional systems of medicine the Indian medicinal plants have been used in successful management of various disease conditions like bronchial asthma, chronic fever, cold,cough, malaria, dysentery, convulsions, diabetes, diarrhoea, arthritis, emetic syndrome, skin diseases, insect bite etc. and in treatment of gastric, hepatic, cardiovascular & immunological disorders (Sen P., 1993).

MATERIALS AND METHOD

Methanol Leave Extract
20 grams of powdered Tulsi leaves were added to 500 ml of methanol and heated and stirred for 3 hour. The extract was treated with 2 grams of charcoal and passed through double filter paper. Repeat the charcoal process twice. Concentrated the extract at 40° and kept at room temperature (Green et al., 2008).

Methanol Stem Extract
Tulsi stem were dried and mashed properly and converted into powder form. 10 grams of Tulsi stem were added to 200 ml of methanol and heated and stirred for 3 hour. Concentrated the extract at 40° and kept at room temperature.

Water Extract
10 mg of Tulsi leave were dissolved in 150 ml of water and kept in water bath for 3 hours at 80° temperature.

Determination of Alkaloid
In the determination of alkaloid content of Tulsi leaves, 5 grams samples leaves powder was taken into 250 ml beaker and 250 ml of 20% CH₃COOH in ethanol was added to it. Magnetic stirrer was used to mix the solution for 10 hr. at room temperature. The solution was filtered and resultant was placed on a hot water bath (60°) until the extract volume turns 1/4th of its initial volume. Concentration NH OH was added drop wise which form thick precipitate. NHOH was added till the formation of the precipitate was complete. The precipitate was collected by filtration, dried in an oven and weighted. (Harborne et al., 1973)

Determination of Flavonoids: In this method 10g of sample was boiled in 50 ml of Reflux condensation for 30 minutes, cooled and filtered. The filtrate was then mixed with equal volume of ethyl acetate. The flavonoids were recovered from the filtrate. (Harborne et al., 1973)

Cold Solvent Extraction
5g of Tulsi leave powder was dissolved in 50ml of methanol in 50ml conical flask & placed on magnetic stirrer for two hours and 45° temperature. After two hour the mixture was cooled at room temperature and the sample was filtered. (Revathy et al.,2011)

In-vitro Antioxidant Study
DPPH Assay
0.1 Mm solution of DPPH (1, 1-Diphenyl –2-picrylhydrazyl) in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentration. Twenty minutes later, the absorbance was measured at 517 nm. The DPPH free radical scavenging activity was calculated using the following (Naama et al. 2010)

Formula: Scavenging ability (%) = (A_{control} - A_{sample} / A_{control}) x 100

Total Antioxidant Assay
Total antioxidant capacity was measured in different concentrations of extract were mixed with 3ml of reagent solution (0.6M sulphuric acid, 28M sodium phosphate and 4M ammonium molybdate), after 90 minutes incubation at 95°C for, sample cool to room temperature and using a digital UV/VIS spectrophotometer (model 371E) absorbance of molybedate (V) formed was measured at 695 nm. (Sharma et al. 2013).

Percentage antioxidant capacity of test compound: Absorbance of test sample/ Absorbance of ascorbic acid X 100

In Vitro Anti-Lipid Peroxidation Assay

The degree of lipid peroxidation was assayed by estimating the TBARS. Silver Nano particles of Tulsi leaves were also prepared.
added to the 10% egg yolk homogenate. Lipid peroxidation was initiated by added 100 µl of (15 mM) ferrous sulphate followed by addition of 0.5 ml of homogenate. After incubation for 1 hour this reaction mixture was mixed with 1.5 ml of 10% TCA. After 10 min of incubation it was centrifuged and 1.5ml supernatant was added in a tube having 1.5 ml of 0.67%TBA (in 50% acetic acid) and placed in a boiling water bath for 30 min. Anti-lipid peroxidation was assessed by using the following formula (Kumar et al., 2013):

Scavenging ability (%) = (A control - A sample / Acontrol) x 100

Preparation of Silver Nano particles

10 mL of aqueous extract of Tulsi leaves was added to 90 mL of silver nitrate solution so as to make its final concentration to 10⁻³ M. The solution was allowed to react at room temperature. Periodic sampling after 30 minutes was carried out to monitor the formation of Silver Nano particles. The qualitative evaluation of reducing potential of aqueous extract of Tulsi leaves was carried out as per the method reported by (Saifuddin et al., 2012.)

RESULT & DISCUSSION

Phytochemical Screening of Ocimum

*Ocimum sanctum* is a popular home remedy for many ailments such as wound, bronchitis, liver diseases, fever, cough, ophthalmic, gastric disorders, genitourinary disorders, skin diseases, various forms of poisoning and Psychosomatic stress disorders (Ashoka et al. 2009). Kayani et. al., 2007 reported that phytochemicals like flavonoids are present in the leaves and stem of most of the wild plants. Alkaloids have been associated with medicinal uses for centuries and one of their biological properties is their cytotoxicity (Singh et al., 2011)

Thin Layer Chromatography

Different solvent system was used to separate various compounds present in the Methanolic extract of *Ocimum sanctum* leaves (table 2). The best separation of compounds was achieved using solvent system consisting of Chloroform: methanol (9:1).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Chloroform</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.5 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>2</td>
<td>9 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>3</td>
<td>8 ml</td>
<td>2 ml</td>
</tr>
<tr>
<td>4</td>
<td>5 ml</td>
<td>5 ml</td>
</tr>
</tbody>
</table>

Table: 4.2: The different solvents are used in TLC method

**In-vitro of free radical scavenging activity of Tulsi**

At the concentration extract showed maximum scavenging of 99.4%, clearly suggesting very strong DPPH free radical scavenging activity of Methanolic extract of *Ocimum* leaves.

Figure 4.3:- Graph showing antioxidant study by DPPH (1-1-diphenyl- 2-picryl hydraazyl) radical scavenging activity.

**Total anti-oxidant activity**

The ability of *Ocimum* sp. to reduce Mo (IV) to Mo (V) was high enough to serve as electron donor and terminate free radical chain reaction.

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Conc. Of extract value</th>
<th>Conc. Of Sulphuric acid value</th>
<th>Conc. Of NS value</th>
<th>Conc. Of ammonium molybdate value</th>
<th>Conc. Value at 695 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.02 ml</td>
<td>1 ml</td>
<td>0.98 ml</td>
<td>1 ml</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.05 ml</td>
<td></td>
<td>0.95 ml</td>
<td>1 ml</td>
<td>0.15</td>
</tr>
<tr>
<td>3</td>
<td>0.1 ml</td>
<td></td>
<td>0.9 ml</td>
<td>1 ml</td>
<td>0.36</td>
</tr>
<tr>
<td>4</td>
<td>0.2 ml</td>
<td></td>
<td>0.6 ml</td>
<td>1 ml</td>
<td>0.62</td>
</tr>
<tr>
<td>5</td>
<td>0.4 ml</td>
<td></td>
<td>0.6 ml</td>
<td>1 ml</td>
<td>1.83</td>
</tr>
</tbody>
</table>

Table 4.4:- Represents absorbance table showed by total antioxidant study of Tulsi

Anti-Lipid peroxidation activity.

Results of our study showed that Methanolic leaf extract possess very strong anti-lipid peroxidation activity. The high anti-lipid peroxidation activity is due to presence of phenolic compounds and high content of flavonoids in leaves which is in correlation with our data regarding flavonoid content in leaves.

Figure 4.5:- graph showing Anti-lipid peroxidation study of Tulsi.

Preparation of Silver Nano Particles

Formation of AgNPs by reduction of silver nitrate during exposure to Tulsi leaf extract can be easily monitored from the change in color of the reaction mixture. As Tulsi possess a potent antioxidant activity, we attribute the reduction process to their presence of high quantity of antioxidants in the leaves extract.

UV-Visible spectroscopic analysis

Synthesis of colloidal silver nanoparticles was initially performed by UV - Visible spectroscopic analysis. In UV – Visible spectrum, a strong peak was observed between 400-420 nm; indicate the presence of silver nanoparticles.
REFERENCE

3. Deshpande RS and Tipnis HP Pesticides 1997; 11: 1-12