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Research Paper

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KEYWORDS :

Antioxidant and Anti- lipid Peroxidation Properties of *Ocimum sanctum*

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ABSTRACT

Present study involves the checking of all below said properties of Tulsi.Tulsi leaves were used as the sample in the research. In-vitro Antioxidant Study by DPPH Assay & Determination Of Flavonoids of Tulsi was done.Determination of Alkaloid content of Tulsi was done both with leaves as well as with stem.In 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.

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 diabtese 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 antidandruff 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 Oscimum, a member of the Lamiaceae family contains 200 sps 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 therapeutic potentials and described as antiasthmatic (Dashemani Shwasaharni) and cough suppressant drugs (Kaphaghna) (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 detemination of alkaloid content of Tulsi leaves, 5 grams samples leaves powder was taken into 250 ml beaker and 250 ml of 20% CH_3COOH 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 (60^0) until the extract volume turns $1/4^{th}$ of its initials volume. Concentration NH_4OH was added drop wise which form thick precipitate. NH_4OH 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 by 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 (%) = $(A_{control} - A_{sample} / A_{control}) \times 100$

Total Antioxidant Assay

Total antioxidant capacity was measured in different concentrations of extract were mixed with 3ml of reagent solution (0.6Msulphuric acid, 28mMsodium phosphate and 4mM 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 by using the standard method with minor modification. Egg yolk homogenate was used as lipid rich medium. A different concentration of the extracts (10-500µl/ml) in water was

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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 (%) = $(A_{control} - A_{sample} / A_{control}) \times 100$

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)

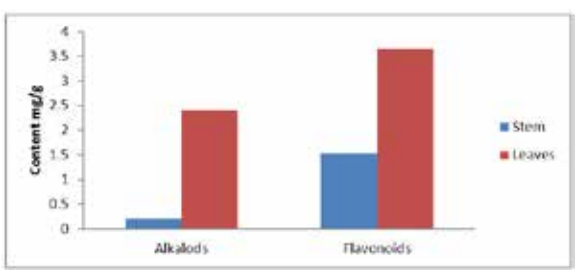

Fig4.1. Alkaloid content and Flavonoid content in leaves and stem

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).

Solvent	Chloroform	Methanol
1	9.5 ml	0.5 ml
2	9 ml	1 ml
3	8 ml	2 ml
4	5 ml	5 ml

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.

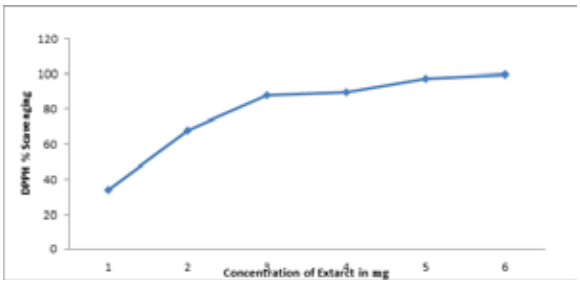

Figure 4.3:- Graph showing antioxidant study by DPPH (1-1-diphenyl- 2-picryl hydrazyl) radical scavenging activity.

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.

Sr.no	Conc.Of extract value	Conc.Of Sulphuric acid value	Conc.Of NS value	Conc.of ammonium molybdate value	Conc. Value at 695 nm
1	0.02 ml	1 ml	0.98ml	1 ml	0.01
2	0.05ml		0.95 ml		0.15
3	0.1ml		0.9 ml		0.36
4	0.2ml		0.8 ml		0.62
5	0.4ml		0.6 ml		1.83

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

Anti-Lipid peroxidation activity.

Results of our study showed that Methanolic leave 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.

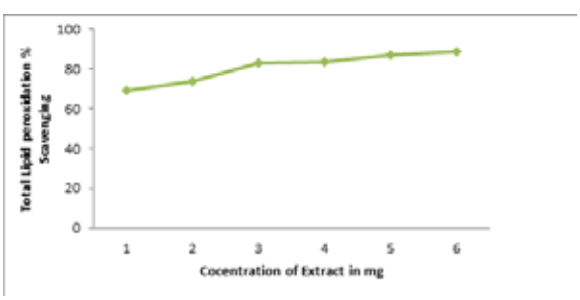

Figure 4.5:- graph showing Anti-lipid peroxidation study of Tulsi.

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

U V-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

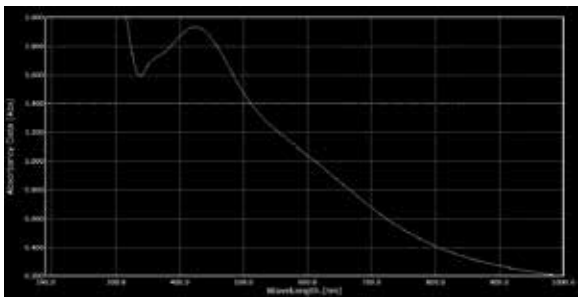

Figure4. 7 showing the presence of silver nanoparticles with the help of uv spectrum

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