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IN-VITRO ANTIOXIDANT ACTIVITY OF OCIMUM SANCTUM LINN

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***Abstract*—Free radical induced oxidative stress is involved in the pathogenesis of various diseases and disorders.Antioxidants play an important role against this oxidative stress to protect our body. The present study was carried out to evaluate the in vitro antioxidant properties of methanol extract of Ocimum Sanctum. in vitro free radical models like, DPPH, nitric oxide, superoxide,hydrogen peroxide and lipid peroxide radical models. The Aim of present study was to estimate the in vitro antioxidant activity of ocimum sanctum.by in vitro antioxidant methods. ascorbic acid is used as standard. Result are produce in the form of graphical structure.concentration of extract, standard, maeketed prepration are increses as well as DPPH scavenging activity and hydrogen peroxide scavenging activity also increases. then conclusion:ascorbic acid are stronger as compare to extact and marketed prepration of ocimum sanctum.**

***Index Terms*—Component, formatting, style, styling, insert.** *(key words)*

I. INTRODUCTION

Living organisms have antioxidant defence systems that protects against oxidative damage by removal or repair of damage molecule.[1] Antioxidants interfere with the oxidative processes by scavenging free radicals chelating free catalytic metals and by acting as electron donors.[2] It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich food and incidence of human disease.[3] The antioxidant action of herbs used in turkey for treating various ailments was evaluated in vitro. Fruits, vegetables and herbs are recommended at present as optimal sources of chemical constituents with antioxidant activity and deactivating free radicals may have beneficially. Antioxidants are copounds that can delay or inhibit the oxidation of lipid or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. They can scavenge free radicals and increase shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food and pharmaceutical products during processing and storage[4].

Many plant contain substantial amount of antioxidants and can be utilized to scavenge the excess free radicals. The protection offered by different edible plant against oxidative stress in several disease has been attributed to various antioxidant and vitamins. Potential antioxidant properties of the dietary phenolic compounds and flavonoids present in various fruits and vegetables have recently been recognized in a number of investigation.[1]

Antioxidant compound in food play an important role as a health protecting factor. Primary source of naturally occurring antioxidants are whole grains, fruits and vegetables. The main characteristics of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species can initiate degenerative diseases.[4].

Plant are one of the most source of medicines. The medicinal are reach in secondary metabolites and essential oil of therapeutic importance. In traditional system of medicine the Indian medicinal plants have been use in successful management of various disease conditions like bronchial asthma, chronic fever, old cough, malaria, dysentery, convulsion, diabetics, diarrhea, arthritis Emetic syndrome and skin diseases, insect bite etc. In Ayurveda Tulsi (Ocimum sanctum L.) has been well documented for its therapeutic potential for various diseases.

A. ANTIOXIDANT

The word antioxidant come from the Greek, anti mean against, plus oxys referring to oxidation.The retardation of oxidation reaction by minute quantities of a certain compound was observed in 1797 by Berthollate and again observed in 1817 by Davy. The first report of use of antioxidant in fats by Deschamps in 1843. These are typically considered under the general category preservation are used to prevents the reaction of certain food constituants.[5]

Antioxidant ‘means a substance when added to food retard or prevents oxidative deterioration of food and does not include sugar, cereal,oils,flours,herb and spices.

Antioxidants are defined by the united states of “Substance used to preserve food by retarding deterioration, rancidity or discoloration due to oxidation.

B. FREE RADICAL

An atom or group of atoms that has at list one unpaired electron and is therefore unstable and highly reactive.oxygen is a highly reactive atom i.e capable of becoming part of potentially damaging molecule commonly called free radical.

Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function.

II. PLANT DETAILS

Plant Used: TULSI

Figure 1: Plant of Ocimum sanctum

Figure 2: Plant extract of Ocimum sanctum

Figure 3: Marketed preparations

A. Antioxidant

The tulsi plant has been tested and found to be a remarkable antioxidant. What this means is it has the ability to slow oxidation in body .the process of oxidation damages cells in the body and may contribute to the worsening of preexisting conditios, as well as effect caused by aging.other antioxidant imclude vitamins E and C.

B. Digestion Aid

The traditional use for tulsi is to help digestion and the organs associated with it. A tea made with tulsi is used to relieve22 stomachache, excessive acidity in the stomach and constipation . The herb is also a remedy for nausea, especially when combined with ginger in tea. Even though tulsi has been used to help relive morning sickness, its use is absolutely not recommended for pregnant women.

C. Immune Booster

Another of the properties of the tulsi plant is that it also acts as a natural immune system booster. This has been one of the plants more common uses throughout history, and it is still one of its more valued bnefits. While generally effective on boosting a persons entire immune system, the tulsi plant appears to provide the largest benefits to the respiratory system and in fighting infecting in that area.

D. Tea

The most common way for people to get the benefits of the tulsi plant is to drink tea made from its leaves.Additionally, many companies are now harvesting several varieties of the tulsi plant and mixing them together to achieve the healthiest results for their customers. Depending on a persons wants, needs,and preferences, chances are good there is a tulsi tea that caters to the benefits that they desire.

E. Preparation of plant extracts

The fresh whole plant of Tulsi was washed with distilled water separately to removed unwanted foreign materials like soil and dusts. After, washed plant material was dried under shade at room temperature without direct exposure of sunrays. It was then coarsely grounded by using mechanical device. The powdered plant material was passed through sieve no 40 and stored in an airtight container for further use. The coarsely powdered plant materials of Tulsi (2000 g) swere extracted separately to exhaustion in a soxhlet apparatus for 72 hours by using Petroleum ether (60 - 80˚C) and methanol (95%) solvent (Merk and Spectrum Chemicals, India) systems. All the extracts were filtered through a cotton plug followed by whattman filter paper (0.1) and then concentrated by using a rotary evaporator at low temperature (40 - 50˚C) and reduced pressure to get 24.4 g and 108.6 g respectively. The extracts were preserved in airtight containers and kept at 4˚C until further use.[5]

F. Introduction : Tulsi

Ayurveda is a system of traditional Hindu medicine which is native to India and is renowned as one of the major systems of alternative and complementary medicine. According to Hindu mythology, Dhanvantari, the physician of the God's, is attributed with the origin of ayurvedic medicine. Ayurveda traces its origin to the Vedas particularly Atharvaveda and it stresses the use of indigenous plant based medicines for the

treatment of diseases [1]. Tulsi "Queen of herbs" is described as sacred and medicinal plant in ancient literature. It is an important symbol of the Hindu religious tradition.[5]

G. Botanical descreption

It is an erect, much branched, fragrant and erected plant attaining a height of about 30-60 cm when mature. Its aromatic leaves are simple, opposite, elliptic, oblong, obtuse or acute with entire or sub serrate or dentate margins, growing up to 5 cm long. The Tulsi flowers are small having purple to reddish color, present in small compact clusters on cylindrical spikes. Stalk less heart-shaped bracts are there at the base of each flower cluster. Sepal cup is not hairy within. Flowers are rarely longer than 5 mm, calyx tube bearded outside near base. Flower tube is hairy. The fruits are small and the seeds yellow to reddish in Colours.[5]

H. Taxonomy

Kingdom:Plantae
Subkingdom:Tracheobionta
Superdivision:Spermatophyta
Division:Magnoliophyta
Class:Magnoliopsida
Subclass:Asteridae
Order:Lamiales
Family:Lamiaceae
Genus:Ocimum
Species : O. sanctum

Synonym: Holy basil ,tulsi ,tulasi ,kali tulsi.

I. Biological source

It conist of the fresh and dried leaves of Ocimum Sanctum and Ocimum Basilicum belonging to family :Lamiaceae.

J. Geographical source

In India, the plant is grown throughout the country from Andaman and Nicobar islands to the Himalayas up to 1800 meters above the sea level [3]. It is also abundantly found in Malaysia, Australia, West Africa and some of the Arab countries. Ocimum sanctum (Linn) is the most prominent species of the genera. The leaves of the plant are considered to be very holy and often form a consistent part of the Hindu spiritual rituals (Tirtha or Prasada). Ocimum sanctum has two varieties i.e. black (Krishna Tulsi) and green (Rama Tulsi), their chemical constituents are similar. Both the varieties also have common medicinal properties.

K. Chemical constituents

It contain volatile oil (0.8%) : Eugenol, nerol,eugenol methylether,caryophyllene,terpinene-4-ol-decyladehyde, camphor, carvacrol, cineole, linalool, oleanolic acid ursolic acid.

L. Phytochemicals

Fresh leaves and stem of Ocimum sanctum extract yielded some phenolic compounds (antioxidants) such as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid, and appreciable quantities of eugeno. The leaves of Ocimum sanctum contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterpine hydrocarbon caryophyllene. Two flavonoids orientin and andvicenin from aqueous leaf extract of Ocimum sanctum have been isolated.

M. Use

Expectorant , bronchitis, stomachic, carminative, stimulant, flavouring agent, Refrigerant and febrifuge, atifertility agent, diaphoretic property , spasmolytic property, antibacterial, insecticidal, antiprotozoal.

III. Medicinal uses of Tulsi

A. Healing Power

The tulsi plant has many medicinal properties. The leaves are a nerve tonic and also sharpen memory.They promote the removal of the catarrhal matter and phlegm from the bronchial tube.The leaves strengthen the stomach and induce copious perspiration.The seed of the plant are mucilaginous.

B. Fever and Common Cold

The leaves of basil are specific for many fevers. During the rainy season, when malariya and dengue fever are widely prevalent,tender leaves, boiled with tea, act as preventive against theses diseases. In case of acute fevers,a decoction of the leaves boiling with powdered cardamom in half a liter of water and mixed with sugar and milk bring down the temperature. The juice of tulsi leaves can be used to bring down fever.Extract of the tulsi leaves in fresh water should be given every 2 to 3 hours. In between one can keep giving sips of cold water. In children, it is every effective in bringing down the temperature.

C. Cough

Tulsi is an important constituents of many Ayurvedic cough syrups and expectorants. It helps to mobilize mucus in bronchitis and asthma. Chewing tulsi leaves relieves cold and flu.

D. Sore Throat

Water Boiled with basil leaves can be taken as drink in case of sore throat. This water can also be used as a gargle.

E. Respiratory Disorder

The herb is useful in the treatment of respiratory system disorder. A decoction of the leaves, with honey and ginger is an effective remedy for bronchitis, asthma, influenza, cough and cold. A decoction of the leaves, colves and common salt also gives immediate relief in case of influenza. They should be boiled in half a litre of water till only half the water is left and add then taken

F. Kidney Stone

Basil has strengthening effect on the kidney. In case of renal stone the juice of basil leaves and honey, if taken regularly for 6 months it will expel them via the urinary tract.

G. Heart Disorder

Basil has a beneficial effects in cardiac disease and the weakness resulting from them. It reduces the level of blood cholesterol.

H. Children Ailments

Common pediatric problems like cough cold,fever,diarrhea and vomiting responds favorably to the juice of basil leaves. If pustules of chiken pox delay their appearance, basil leaves taken with saffron will hasten them.

I. Stress

Basil leaves are regarded as an adaptogen or anti-stress agent. Recent studies have shown that the leaves afford signification protection against stress. Even healthy persons can chew 12 leaves of basil, twice a day, to prevent stress. It purifies blood and helps prevent several common elements.

J. Mouth Infection

The leaves are quits effective for the ulcer and infection in the mouth. A few leaves chewed will cure these condition.

Insect Bites: The herb is a prophylactic or preventive and curative for insect stings or bites. A teaspoonful of the juice of the leaves is taken and is repeated after a few hours. Fresh juice must also be applicable to the affected parts. A paste of fresh root is also effective in case of fresh root is also effective in case of bites of insects and leeched.

K. Skin Disorders

Applied locally,basil juice is beneficial in the treatment of ringworm and other skin diseases. It has also been tried successfully by some naturopaths in the treatment of leukoderma.

L. Teeth Disorders

The herb is useful in teeth disorders.its leaves,dried in the sun and powered, can be used for brushing teeth. It can also be mixed with mustered oil to make a paste and used as tooth paste. This is very good for

M.Headaches

Basil makes a good medicine for headache. A decoction of the leaves mixed can be given for this disorder. Pounded leaves mixed with sandalwood paste can also be applied on the forehead for getting relief from heat, headache, and for providing coolness in general.

N. Eye disorders

Eye problems like sore eyes and night blindness can effectively be relieved with the regular use of tulsi extracts.

O. Breathing Problems

Extracts from this plant often used to relieve respiratory problems like asthma and bronchitis.

P. Sharpen Memory

Extract from the leaves of this herb act as a tonic for the nerves and help sharpen the memory too.

IV. IN VITRO ANTIOXIDANT METHODS

- Determination of DPPH Radical Scavenging Activity.
- Determination of Hydrogen Peroxide (H_2O_2) Scavenging Activity
- Determination of Nitric Oxide (NO) Scavenging Activity.
- Determination of Superoxide (SO) Radical Scavenging Activity
- Determination of Lipid Peroxide (LPO) Radical Scavenging Activity.
- Determination of Reductive Ability.
- Determination of Total Phenolic Content.
- Determination of Total Flavonoid Content.

V. IN VITRO ANTIOXIDANT ACTIVITY

A. Determination of DPPH Radical Scavenging Activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was measured using the method of Cotelle et al[16] with some modifications. 3 ml of reaction mixture containing 0.2 ml of DPPH (100 mM in methanol) and 2.8 ml of test or standard solution of various concentrations was incubated at 37°C for 30 min and absorbance of the resulting solution was measured at 517 nm using Beckman model DU-40 spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the formula.

B. Determination of Hydrogen Peroxide (H_2O_2) Scavenging Activity

The hydrogen peroxide scavenging ability of the extract was determined according to the method of Ruch et al[19]. A solution of H2O2 (40 mM) was prepared in phosphate buffer (pH 7.4). Extract or standards (of different concentrations) in phosphate buffer (3.4 ml) was added to the H2O2 solution (0.6 ml, 40 mM). The absorbance of the reaction mixture was recorded at 230 nm after 10 min against a blank solution of phosphate buffer. Percentage of H2O2 scavenging was calculated using the above formula.[9]

C. Determination of Nitric Oxide (NO) Scavenging Activity

At physiological pH, sodium nitroprusside generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be measured by the Griess reaction[17]. 1ml of 10 mM sodium nitroprusside was mixed with 1 ml of test or standard solution of different concentrations in phosphate buffer (pH7.4) and the mixture was incubated at 25°C for 150 min. From the incubated mixture, 1 ml was taken out and 1 ml of Griess' reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was

added to it. Absorbance of the chromophore formed by the diazotization of nitrite with sulfanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was read at 546 nm and percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.[9]

D. Determination of Superoxide (SO) Radical Scavenging Activity

Superoxide anion scavenging activity was measured according to the method of Robak and Gryglewski[18] with some modifications. All the solutions were prepared in 100 mM phosphate buffer (pH 7.4). 1ml of nitroblue tetrazolium (NBT, 156 μM), 1 ml of reduced nicotinamide adenine dinucleotide (NADH, 468 μM) and 3 ml of test/ standard solution were mixed. The reaction was initiated by adding 100 μl of phenazine methosulphate (PMS, 60 μM). The reaction mixture was incubated at 25◦C for 5 min, Free Radicals and Antioxidants 44 Vol 1, Issue 1, Jan-Mar, 2011 In vitro antioxidant activity of C.maxima followed by measurement of absorbance at 560 nm. The percentage inhibition was calculated from the above formula.[9]

E. Determination of Lipid Peroxide (LPO) Radical Scavenging Activity

Rat liver homogenate was used as the source of polyunsaturated fatty acids for determining the extent of lipid peroxidation. Reaction mixture (0.5 ml) containing rat liver homogenate (0.1 ml, 25% w/v) in Tris–HCl buffer (40 mM, pH 7.0), KCl (30 mM), ferrous ion (0.16 mM) and ascorbic acid (0.06 mM) were incubated at 37°C for 1 h in the presence or absence of the extracts or standards. The lipid peroxide formed was measured according to the method of Ohkawa et al[20]. Incubation mixtures were treated with sodium dodecyl sulphate (SDS; 8.1%, 0.2ml), thiobarbituric acid (TBA; 0.8%, 1.5 ml) and acetic acid (20%, 1.5ml). The total volume was then made up to 4 ml with distilled water and kept on water bath for 30 min. After cooling, 1 ml of distilled water and 5 ml of a mixture of n-butanol and pyridine (15:1 v/v) were added and centrifuged at 4000 rpm for 10 min. The absorbance of the organic layer, containing the colored TBA-MDA complex, was measured at 532 nm. The percentage inhibition of lipid peroxidation was determined by comparing the results of the test compound with those of the control, using the formula mentioned above.[9]

F. Determination of Reductive Ability

Reducing power of the extract and the standard was determined on the basis of the ability of their antioxidant principles to form colored complex with potassium ferricyanide, trichloro acetic acid (TCA) and FeCl3, which is measured at 700 nm[21]. 1 ml of different concentrations of the extract or standard solution was mixed with potassium ferricyanide (2.5 ml, 1%) and 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50°C for 20 min. 2.5 ml TCA (10%) was added to it and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was taken out and to this 2.5 ml water and 0.5 ml FeCl3 (0.1%) were added and absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power.[9]

G. Determination of Total Phenolic Content

The amount of total phenolic compounds in MECM was determined using Folin-Ciocalteu's reagent and sodium carbonate solution and the absorbence was measured at 760 nm[22]. A calibration curve of standard pyrocatechol was prepared and the results were expressed as mg of pyrocatechol equivalents /g of dry extract.

H. Determination of Total Flavonoid Content

The total flavonoid content of MECM was determined spectrophotometrically[23]. Briefly 0.5 ml of 2% aluminium chloride in ethanol was mixed with same volume of extract (1.0 mg/ml). Absorption readings at 415 nm were taken after 1 h against a blank (ethanol). The total flavonoid content was determined using a standard curve with quercetin (0-50 mg/L). The mean of three readings was used and expressed as mg of quercetin equivalents/ g of dry extract.

VI. EXPERIMENTAL WORK

AIM

To estimate In -vitro antioxidant activity of Ocimum Sanctum.

Material

Chemicals are issued from the laboratory. All the chemicals and reagents are of analytical grade.

Chemicals

DPPH solution , methanol, Ascorbic acid, Ocimum Sanctum extract, Markeded Product, Nitric acid, phosphate buffer (7.4).

Apparatus

Test tube, Measuring cylinder, Volumetric flask, Beaker, Test tube stand, Pippete etc.

Figure No.1 List of Instrument

Instrument	Company Name
UV –Visible Spectrophotometer	Shimadzu
Colorimeter	Aparna
Digital weighing balance	Dolphin

VII. METHODS

In-Vitro Antioxidant Activity

1) Determination of DPPH Radical Scavenging Activity

- 0.1M solution of DPPH in ethanol was prepared.
- This solution (1 ml) was added to 3ml of different extract in ethanol at a different concentration (10,20,40,60,80,100 µg/ml).
- Here, only those extract are used which are soluble in ethanol and their various concentration were prepared by dilution method.
- The mixture was shakend vigorously and stand to room temperature for 30 min.
- Absorbance measured at 517nm by using spectrophotometer.
- Reference standard solution being used was ascorbic acid

2) Determination of Hydrogen Peroxide (H2O2) Scavenging Activity :

- The hydrogen peroxide scavenging ability of the extract was determined according to the method of Rich et al.
- A solution of H2O2 (40 mm) was prepared in phosphate buffer (pH 7.4).
- Extract or standards (of different concentrations) in phosphate buffer (3.4 ml) was added to the H2O2 solution (0.6 ml, 40 mm).
- The absorbance of the reaction mixture was recorded at 230 nm after 10 min against a blank solution of phosphate buffer.

VIII. RESULT AND DISUCSSION

Determination DPPH Radical Scavenging Activity

% Inhibition = (Absorbance of control - absorbance of test) × 100/ Absorbance of control

TABLE NO. 1 Observation of DPPH Radical Scavenging Activity

Sample	**Percent inhibition**					
	10µg/ml	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml
Extract (Ocimum Sanctum)	57.42%	63.86%	67.32%	69.30%	75.74%	80.19%
Markeded Product	44.05%	49.50%	57.92%	65.82%	69.86%	72.77%
ASCORBIC ACID	78.71%	80.69%	82.17%	85.17%	89.10%	89.60%

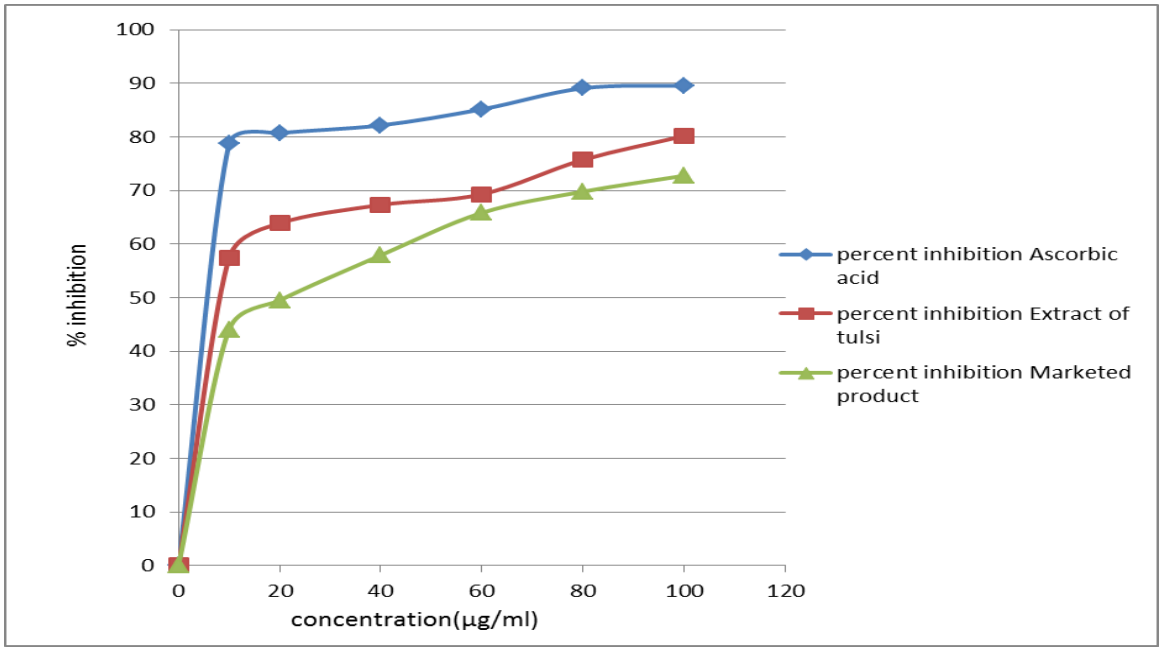

Figure 1 Concentration vs. percent inhibition

IX. Discussion

The DPPH scavenging activity of extract, standard marketed antioxidant & ascorbic acid was given in above table. It is revealed that as concentration extract, standard marketed antioxidant & ascorbic acid was increase, the DPPH scavenging activity was also increases.

DPPH radical scavenging assay is one of the most widely used methods for screening of antioxidant property of plant products. DPPH is a stable nitrogen centered free radical and can easily abstract an electron or hydrogen radical from the suitable reducing agents to become a stable diamagnetic molecule. The unpaired electron of DPPH thus gets paired off forming the corresponding non-radical hydrazine. The radical scavenging property of the sample was determined by measuring the decrease in absorbance of DPPH.

Determination of Hydrogen Peroxide (H_2O_2) Scavenging Activity

Percentage inhibition = (Absorbance of control - absorbance of test) × 100/ Absorbance of control

TABLE NO. 2 Observation of Hydrogen Peroxide Scavenging Activity

Sample	Percent Inhibition					
	10µg/ml	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml
Extract(Ocimum Sanctum)	18.99%	22.86%	25.19%	31.20%	37.40%	39.92%
Marketed product	13.56%	16.08%	20.15%	24.41%	29.84%	35.27%
Ascorbic acid	25.19%	29.26%	31.97%	34.24%	36.24%	37.79%

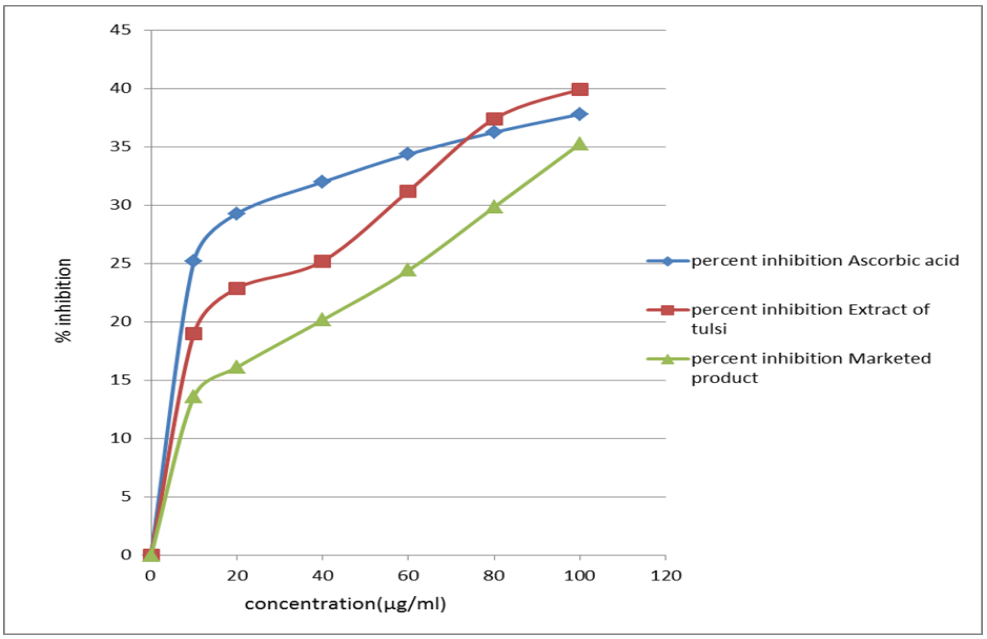

Figure.2: Concentration vs. percent inhibition

X. DISCUSSION

The H2O2 scavenging activity of extract, standard marketed antioxidant & ascorbic acid was given in above table. It is revealed that as concentration extract, standard marketed antioxidant & ascorbic acid was increase, the H2O2 scavenging activity was also increases.

XI. CONCLUSION

Ocimum sanctum plant is used as antiseptic, febrifuge, tonic, emetic & Antioxidant property. The antioxidant capacity of Ocimum Sanctum extract showed stronger activity. The Ocimum Sanctum extract show stronger antioxidant activity as compared to ascorbic acid. These antioxidant activity depended on concentration of sample. Mareketed preparation also shows greater antioxidant activity. It is believed that detection of natural antioxidant source & proper consumption of them in daily diet or use of isolated compounds in clinical practice would be beneficial for healthy life.

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