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ISOLATION AND CHARACTERIZATION OF *OCIMUM SANCTUM* SEEDS FOR ITS ANTIOXIDANT ACTIVITY

Priya Sharma*, Dr. Naresh Singh Gill *, Amandeep Kaur

Department of Pharmaceutical Chemistry, Rayat Institute of Pharmacy, Railmajra

ABSTRACT :- The sacred basil Ocimum sanctum (Tulsi) , is renowned for its religious and spiritual sanctity, as well as for its important role in the traditional Ayurvedic and Unani system of holistic health and herbal medicine of the East.belongs to family lamiaceae. The plant has been famous for its curative properties and has been put to use for treatment of various ailments suchlike ulcer, bronchitis, jaundice, fever, cold and cough , arthritis and alimentary disorders. The present investigation was intented to evaluate the preliminary phytochemical characters as well as antioxidant properties of the plant. Phytochemical studies promotes new discovery for the synthesis of more potent drugs. Antioxidant activity of plant extract was evaluated by Hydrogen peroxide (H2O2) and Nitric Oxide (NO) method. Alkaloids , Flavanoids and triterpenoids were detected in the plant, With the increase in concentration of seed extract, the antioxidant activity increased proportionally to the maximum activity of 70.84% at 125µg/mL and 80.56% at 125µg/mL with H2O2 and NO respectively.

KEYWORDS :- Phytochemicals, Ocimum sanctum ,Flavanoids. Triterpenoids.

INTRODUCTION

Traditionally, O. sanctum L. is taken in many forms, as herbal tea, dried power or fresh leaf. For centuries, the dried leaves of Tulsi have been mixed with stored grains to repel insects (Warrier PK.1995.) Plants are potent biochemists and have been components of phytomedicine since times immemorial. A rich heritage of knowledge on preventive and curative medicines was available in ancient scholastic work included in the Atharvaveda (an Indian religious book), Ayurveda (Indian traditional system of medicine) and so on. It is an erect, much branched sub-shrub 30-60 cm tall, with simple opposite green or purple leaves and stems with a aromatic odour, the branches with soft white hairs and leaves with blades elliptic to elliptic oblong and acute at

apex. Flowers terminal, slender racemes or panicles. Fruit with nutlets purple, green to brown , broadly ellipsoid 0.8 to 1.2 mm long (Kiritikar K R. Basu, B. D.1975). Tulsi varieties readily grow wild in many areas of Asia and Africa. It is also abundantly found in Malaysia, Australia and some of the Arab countries. Ocimum species thrive well in a variety of soils and climatic conditions. Monoterpene are obtained from the the volatile oils such as, camphene, myrcene, sabinene, in which some mono terpene produced oxygen such as linalool, borneol . Phytochemical analysis of this medicinal herb can identify the nature of compounds present in the extract of ocimum sanctum.More than 60 chemical compounds have been reported from O. sanctum, including phenolics, flavonoids, phenyl propanoids, terpenoids, fatty acid derivatives, essential oil, fixed oil, and steroids. The pharmacological activities of O. sanctum compounds reflect their medicinal importance and in the standardization of medicinal products.The aromatic volatile oil mainly constitutes phenols, terpenes and aldehydes. Flowers are bracteate, pedicellate, complete, hermaphrodite, hypogynous, zygomorphic, pentamerous. Androecium; stemens 4, epipetlous, didynamous, posterior stemen absent, dithecous, dorsifixed, introse. Gynoecium . The Bicarpellary, syncarpous,superior, gynobasic style, axile plcentation, hypogynous, necter secreting disc present. Fruit is schizocarpic, having four nutlets. The fruits are small and the seeds are reddish-yellow in colour (Chopra RN. 1956). Tulsi plant presence symbolizes the religious bent of a Hindu family. A Hindu household is considered incomplete if it doesn't have a tulsi plant in the courtyard. Many families have the tulsi planted in a specially built structure, which has images of deities installed on all four sides, and an alcove for a small earthen oil lamp .O. sanctum 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 (Kothari. S. K. 2005).

MATERIAL AND METHODS

COLLECTION OF PLANT MATERIAL

The seeds of Ocimum Sanctum were collected in the month of October and November from the city Rupnagar , Punjab (India) . After that , the clean and healthy seeds are seprated for future procedure . The healthy seeds were selected for authentication from NISCAIR (National Institute of Science Communication and Information Resources) New Delhi.

EXTRACTION OF *OCIMUM SANCTUM* SEEDS

The healthy seeds of Ocimum sanctum were selected and shade dried. The dried seeds were weighed and subjected to cold maceration for 3 days with methanol. The extraction procedure is given below:

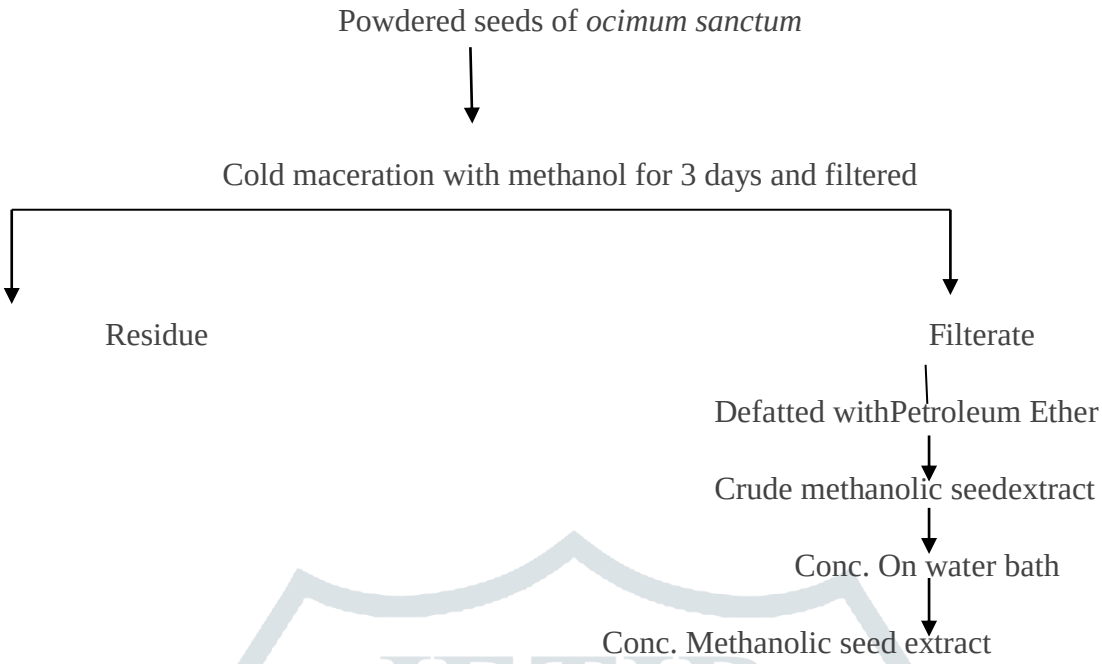

Figure 1: Flow chart for seed's extract

PHYTOCHEMICAL SCREENING OF EXTRACT

The Photochemical screening of Pedalium murex seeds extract showed the presence of various chemical constituents like flavonoids, alkaloids, triterpenoids, cardiac glycosides.

ANTIOXIDANT ACTIVITY

In vitro evaluation of free radical scavenging activities is done by following 2 methods:

- H2O2 Method
- NO Method

Quantitative Evaluation of H2O2 Free radical scavenging Activity

Requirements

Chemicals and Reagents :- H2O2 (40mm) , Phosphate buffer(0.1m), Ascorbic acid

Blank :- Methanol

Control :- H2O2 solution The Hydrogen Peroxide Radical Scavenging Activity of Methanolic extract of ocimum sanctum seeds were screened for their possible antioxidant activity by H2O2 Free radical scavenging assay. In H2O2 assay, percentage inhibition in ocimum sanctum seeds extract was in range between 22.34 to 70.84. A solution of Hydrogen peroxide was prepared in 0.1 M phosphate buffer 1ml of each methanolic extract of ocimum sanctum seeds at different concentrations (10- 125ml) was added to 0.6 ml of 40 M hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank containing phosphate buffer without hydrogen peroxide.

The percentage scavenging of hydrogen peroxide of plant extract and reference standard ascorbic acid was calculated using the following formula.

$$\text{Scavenging } (H_2O_2) = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Mixture of 0.1 M Dipotassium hydrogen phosphate and Potassium Dihydrogen phosphate were used as Buffer.

Quantitative Evaluation of NO Free radical scavenging Activity

Requirements :- Reagents

- 10 mM sodium nitroprusside
- Phosphate buffered saline pH 7.4
- 2% sulphanilamide in ortho-phosphoric acid
- naphthylethylenediamine dihydrochloride

Procedure: To Iml of sodium nitroprusside, 2.5 ml, phosphate buffered saline pH 7.4 was added and mixed with 1 ml of extract at various concentrations (ug/ml), then the mixture was incubated at 25° C for 30 minutes. From the incubated mixture 1.5 ml was ken. To it, Iml of sulphanilamide in phosphoric acid and 0.5 ml of naphthylethylenediamine dihydrochloride were added and the absorbance was measured at 544 and Ascorbic acid was used as a standard. The percentage inhibition of nitric oxide radical generated was calculated by using the following formula:

$$\text{Scavenging (NO)} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

RESULT AND DISCUSSIONS

The phytochemical characteristics of medicinal plant tested were summarized in the table. The results revealed the presence of medically active compounds in the plant studied. From the table, it could be seen that alkaloids, triterpenoids, flavonoids, cardiac glycosides and volatile oils were present in the plant.

Results of Phytochemical screening of methanolic extract

S.NO.	PHYTOCONSTITUENTS	RESULTS
1.	ALKALOIDS	++
2.	FLAVANOIDS	+++
3.	CARDIAC GLYCOSIDES	++
4.	SAPONIN GLYCOSIDES	-
5.	CARBOHYDRATES	-
6.	STEROIDS AND TRITERPENOIDS	+++
7.	PROTEINS	+
8.	STEROLS	+
9.	TANNINS	++
10.	VOLATILE OIL	++

(-) indicates absence of chemical constituents / (+) indicates presence of chemical constituents / (+++) indicates higher cntent of chemical constituents.

IN VITRO ANTIOXIDANT ACTIVITY

Quantitative Estimation of Antioxidant Activity using Hydrogen Peroxide Method

The Hydrogen Peroxide (H2O2) method is based on the decrease in absorbance of H2O2 following reduction of H2O2 by the antioxidant compound . The Hydrogen Peroxide (H2O2) is an oxidant that is being formed continuously in living tissues as a result os several metabolic processes.

The measurement of H_2O_2 scavenging activity is one of the useful method for determining the ability of antioxidant.

% Hydrogen peroxide radical scavenging activity= $A_0 - A / A_0 \times 100$

A_0 = control absorbance (blank) A_1 = absorbance of test/ std. sample

Table 1 : Hydrogen peroxide scavenging activity of *Ocimum sanctum* seeds

S.NO,	Concentration (ml)	%scavenging Activity (Test)	%scavenging Activity (Standard)
1.	50	45.03±0.002	48.91±0.001
2.	75	59.28±0.001	63.34±0.001
3.	100	66.03±0.002	69.67±0.002
4.	125	70.84±0.001	73.84±0.003

The methanolic extract of Ocimum sanctum seeds exhibited H_2O_2 Scavenging activity, with the increase in concentration of seed extract , the antioxidant activity increased proportionally with the maximum activity of 70.84 % at 125ml. The absorption decreased proportionally with the increase in the concentration of extract.

The percentage inhibition of test and standard samples was calculated by subtracting their absorptions from the absorption of control sample according to formula :-

% Hydrogen peroxide radical scavenging activity= $A_0 - A / A_0 \times 100$

A_0 = control absorbance (blank)

A = absorbance of test/ std. sample

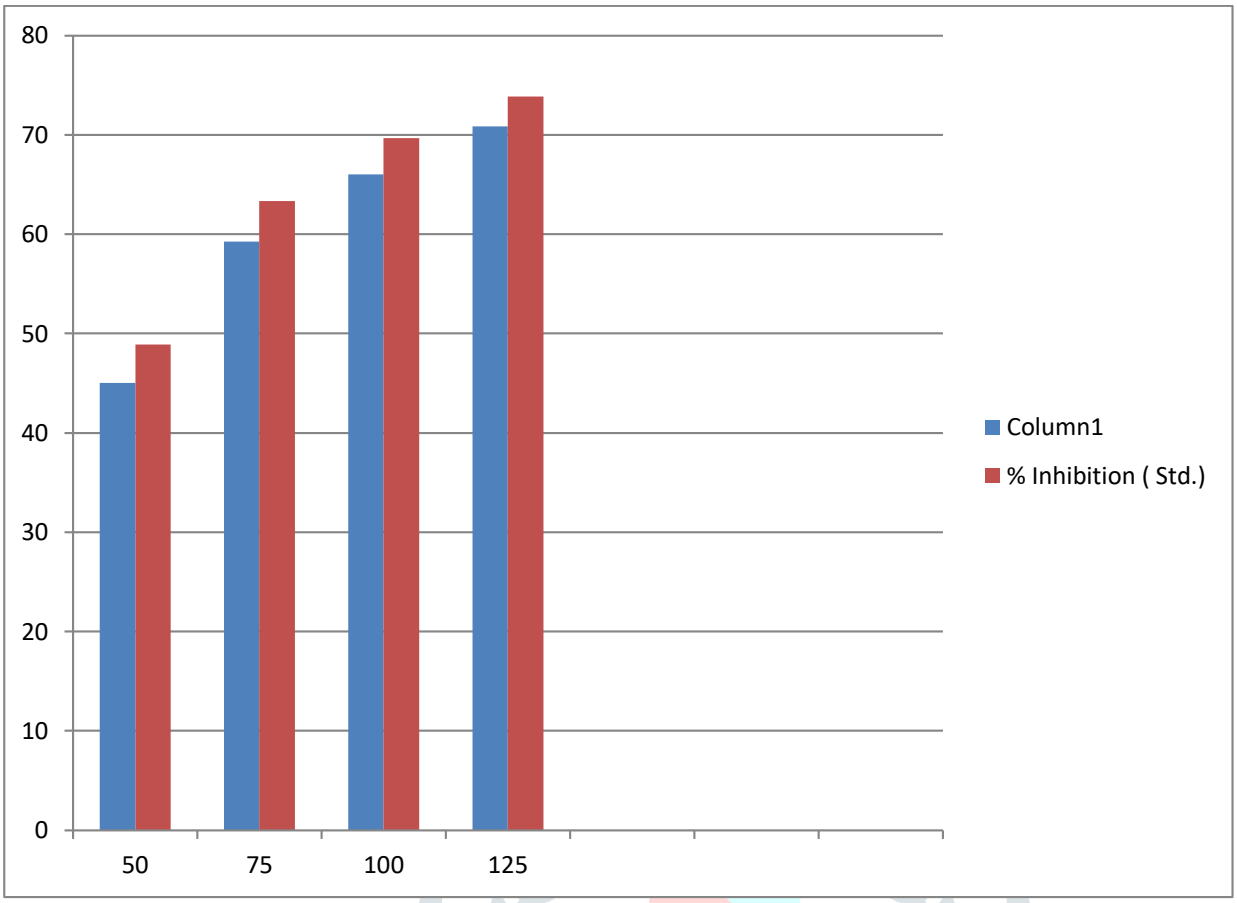

Figure 2 : Graphical representation of H_2O_2 Scavenging Activity

Quantitative Estimation of Antioxidant Activity using Nitric Oxide Scavenging Method

The Nitric Oxide scavenging method is based on quantitative determination of nitrite and nitrate in compound. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interact with oxygen to produce nitrite ions, which can be determined by the use of the Griss Illosvoy reaction. The nitrite ions produced diazotizes sulphanilamide and then the diazonium salt reacts with N, N Naphthyl ethylene diamine dihydrochloride to give a pink colour chromophore which has a maximum absorption at 544 nm.

Table 2 : Nitric Oxide scavenging activity of *Ocimum sanctum* seeds

S.NO,	Concentration (ml)	%scavenging Activity (Test)	%scavenging Activity (Standard)
1.	50	62.25	67.70
2.	75	66.62	71.46
3.	100	74.46	77.23
4.	125	80.56	86.12

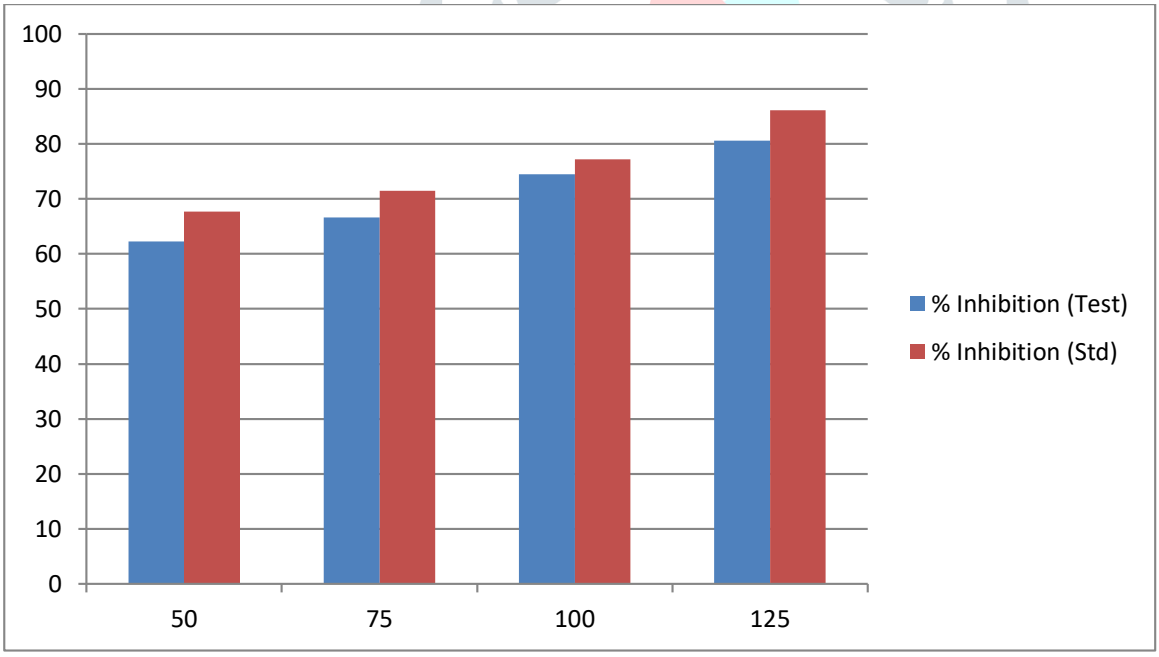

Figure 3 : Graphical representation of NO Scavenging Activity

CHARACTERIZATION OF THE ISOLATED FRACTION BY IR AND NMR ANALYSIS

IR SPECTRA : Infrared spectra (IR) of Toluene: Ethyl acetate: Formic acid: Glacial acetic acid (5:5:0.1:1) fraction of ocimum sanctum seed's extract showed major peaks corresponding to alcoholic (OH), alkanes (C-H), and amide groups respectively.

IR Spectra (KBr) : It showed the presence of peaks at 33.25 (O-H stretching), 2944 (C-H stretch), 2924 (CH stretching), 1662 (CH_2 Bending), 1375 (CH_3 Bending), 1240 (C-OH Bending).

NMR SPECTRA: The NMR spectra were recorded at 400 Hz using Dimethyl sulphoxide (DMSO) as the solvent . Different chemical shifts were recorded characteristic to the compound.

^{1}H NMR (Proton NMR) Chemical shifts :

ð0.83-1.22ppm (methyl group) , ð0.85-0.90 (Aliphatic proton), ð1.54-3.13ppm (Methylene proton) ,ð2ppm (alcoholic proton), ð0.97PPM (up field chemical shift), ð2.03-3.31ppm, ð7.25 (downfield chemical shift).

^{13}C-NMR (Carbon NMR):

δ 14.0 (1C,), 22.6 (1C,), 25.6 (1C,), 25.8-25.9 (2C) 25.8 (s), 25.8 (s), 26.4-26.7 (4C) 26.5 (s), 26.5 (s), 26.6 (s), 26.6 (s), 27.3 (1C), 27.6-27.7 (2C, 27.6), 27.6 (s), 29.3 (1C), 29.6 (1C), 31.8 (1C), 35.8-35.8 (3C) 35.8 (s), 35.8 (s), 35.8 (s), 36.5 (1C), 39.1 (1C), 39.9-39.9 (2C) 39.9 (s), 39.9 (s), 71.7 (4C).

The NMR and IR spectral analysis showed that the compound isolated from Ocimum sanctum methanolic seed extract may be Triterpenoid and steroidal in nature.

Further studies may be required to confirm the exact structure of compound.

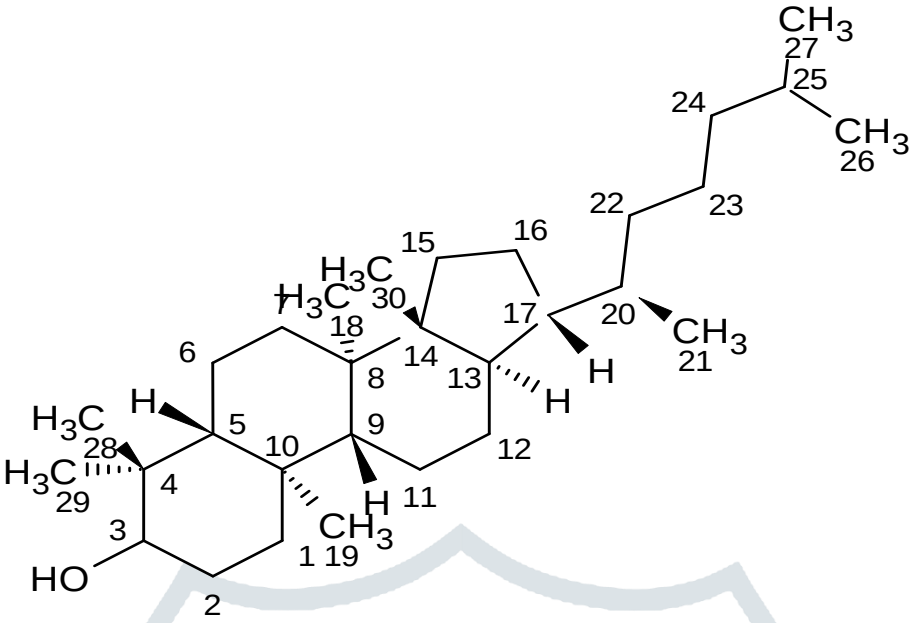

Figure 4 : Hypothetical structure of Isolated Compound

CONCLUSION

In conclusion, the present investigation on exploring the Therapeutic potential of Ocimum Sanctum revealed that this herb/plant is rich source of Antioxidants. This study also provided a scientific support to the effectiveness of Ocimum Sanctum seeds in free radical mediated diseases. These could be used as natural preservatives and in the development of nutraceuticals formulations to overcome the adverse effects of synthetic compounds.In this research, Phytochemical studies on Ocimum Sanctum seeds extract showed the presence of triterpenoids in higher amount than tannins. One compound was isolated from it methanolic seeds extract using TLC and it was characterized by using NMR and IR technique that the isolated compound contained a triterpenoids and flavanoids.

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