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In Vitro Anti oxidant activity of Hydro-alcoholic extract of *Origanum vulgare*

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Abstract

Present study deals with the *In vitro* Anti oxidant activity of hydro alcoholic extract of *Origanum vulgare* commonly known as oregano belongs to the family Lamiaceae. Hydro alcoholic extract of *Origanum vulgare* was subjected to *In vitro* antioxidant activity screening models such as DPPH, ABTS radical scavenging activity, inhibition of Lipid peroxidation where Gallic acid, Butylated Hydroxy Toulene (BHT) and Ascorbic acid were used as the standards. In all the models studied, hydro alcoholic extract of *Origanum vulgare* showed nearly equal activities to Standards used. In conclusion, the present study approved that the *O.vulgaris* extract have promising *In- vitro* antioxidant activity.

Key words: *Origanum vulgare*, DPPH, ABTS, Lipid peroxidation

Introduction

Medicinal plants are of great value for the treatment and cure of diseases. Over the years, scientific research has expanded our knowledge of the chemical effects and composition of the active constituents, which determine the medicinal properties of plant. Chemical diversity in natural product is an immensely rich source of new pharmaceuticals cosmetics, agrochemicals and other economically important chemicals. Natural products as a basis for new drugs, have great promise and it is gratifying to note that the World Health Organization have shown an abiding interest in plant derived medicines, described in the folklore of various countries [(1).]

Antioxidants have been reported to prevent oxidative damage by free radical and reactive oxygen species and may prevent the occurrence of diseases, cancer and aging. It can interfere with the oxidative process by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers. Plant and plant products are being used as a source of medicine since long. Novel natural antioxidants from some plants have been extensively studied in the past few years for their antioxidant and radical scavenging properties. The non enzymatic antioxidants which act as scavengers are glutathione, vit. A, vit. E and vit. C [(2)].

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Origanum vulgare commonly known as oregano belongs to the Lamiaceae family which is native to warm temperate environments from Eurasia to the Mediterranean region. Oregano is used in treatment of nausea, as an anti-inflammatory agent , a pain remedy, a warming remedy and antioxidant herb . The main pharmacological effects of *O. vulgaris* are being antioxidant, anti-thrombin and potent antihypertensive through maintaining renal function, creatinine clearance and inhibiting mechanical forces acting on arterial wall so preventing excess hardening and thickening. Urinary stone formation is one of the early decades known diseases [(3).]

Material and Methods

Collection of Plant Material: - The fresh whole herb of *Origanum vulgare*was collected from local market Indore. The identity of the herb was confirmed by Department of Pharmacognosy, Lakshmi Narain College of Pharmacy, Indore A voucher specimen was kept in the department for future reference.

Preparation of the hydro alcoholic extract:

Dried leaves and the flowering branches of *Origanum vulgare* were homogenized to a fine powder which were extracted twice with 70% ethanol for 24 h at room temperature.The extract was filtered, then concentrated over the water bath, brought to dryness under vacuum, until use [(4).]

DPPH Radical Scavenging Activity [(5)]: 15 mg of DPPH was dissolved in 10 ml of methanol. 75µl of this solution was taken and the final volume was adjusted to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 75µl of

DPPH was added to a mixture of methanol and 50 µl of extract. The final volume was adjusted to 3 ml. Decrease in absorbance of the DPPH was measured 517 nm

ABTS Radical Scavenging Activity [6]: ABTS 2mM and Potassium per sulphate 70mM were prepared in distilled water (0.0548g in 50 ml and 0.0189g in 1ml respectively). 200ml of Potassium per sulphate and 50 ml of ABTS were mixed and used after 2 hrs. To 0.5 ml of various concentrations of the extracts, 0.3 ml of ABTS radical cation and 1.7 ml of Phosphate buffer, pH 7.4 was added. For control, instead of extract, methanol for alcoholic extract and water for aqueous extract were taken. The absorbance was measured at 734 nm.

Lipid Peroxidation Assay [7]: 15% w/v Trichloroacetic acid, 0.375%w/v thiobarbituric acid and 0.25N Hydrochloric acid were mixed to form the stock Thiobarbituric acid (TBA)- Tri-chloro acetic acid (TCA)- HCl reagent. This solution was mildly heated to assist the dissolution of TBA]. Albino rats (180-200g) of either sex were used for the study. After decapitation, the brain was removed carefully. The tissue was immediately weighed and homogenated with cold 1.15%w/v KCl to make 10%v/v homogenate. The homogenate (0.5ml) was added to 1 ml of various concentrations of the extracts. Then the mixture was incubated for 30 min. The per-oxidation was terminated by the addition of 2 ml of TBA-TCA –HCl reagent. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the supernatant was measured at 535 nm. The % inhibition of various radicals was calculated by comparing the results of the test with those of control using the formula.

$$\% \text{ Scavenging} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

committee approved the use of animals for lipid peroxidation assay (IAEC/SCOPE/07-08/01). All experiments were performed in triplicate and the results averaged. Linear regression analysis was used to calculate the IC50 values .

Results and Discussion

Several concentrations ranging from 50-150 µg /ml of extract of *Origanum vulgare* were compared for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the extracts in a concentration dependent manner up to the given concentration in all the models.

DPPH Radical Scavenging Activity [8,9]

The in vitro antioxidant assay performed on this plant reveals significant antioxidant potential compared with gallic acid as a standard. The DPPH radical scavenging activity of hydro alcoholic extract of *Origanum vulgare* are shown in table 1.1. This hydro alcoholic extract showed encouraging response in quenching DPPH radical with an IC_{50} value of 97.68µg/ml. The *in vitro* study carried out on this radical is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH . This radical reacts with suitable reducing agents, the electrons become paired off and the solution loses color stoichiometrically depending on the number of electrons taken up . From the present result, it may be concluded that hydroalcholic *Origanum vulgare.* reduce the radical to the corresponding hydrazine when they react with the hydrogen donors in the antioxidant principles.

ABTS Radical Scavenging Activity of [10,11, 12]

The ABTS radical scavenging activity of hydroalcoholic extract of *Origanum vulgare.* is shown in table 1.2.This activity is comparable with that of BHT, the standard antioxidant used in this study.The hydroalcoholic extract showed encouraging response in quenching ABTS radical with an IC_{50} value of 85.48µg/ml. The ABTS chemistry involves direct generation of ABTS radical mono cation with no involvement of any intermediary radical. It is a decolorization assay, thus the radical cation is performed prior to the addition of antioxidant test system, rather than the generation of the radical taking place continuously in presence of the antioxidant

Lipid Per-oxidation Activity of *Oregano* [13,14,15]

The Lipid Per-oxidation activity hydro alcoholic extract of *Origanum vulgare* is shown in table 1.3 . The hydro alcoholic extract showed encouraging response in quenching the lipids with an IC_{50} value of 93.15µg/ml. In this study, lipid peroxidation was induced *in vitro* and the extract showed concentration dependent prevention towards generation of lipid peroxides.

Conclusion

The present study proved that hydroalcoholic extract of *Origanum vulgare* to be nearly potent to the standard drug used thereby justifying its traditional claims and its use in the present day system of medicine.

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Table 1.1: DPPH Radical Scavenging Activity of *Origanum vulgare*

concentration(µg/ml)	% scavenging (hydroalcholic extract)	% scavenging (std)
50	16.85 ± 1.89	26.03 ± 1.04
75	29.68 ± 1.41	42.90 ± 0.93
100	55.53 ± 3.44	56.62 ± 0.86
125	71.45 ± 4.56	68.63 ± 0.33
150	84.17 ± 3.69	77.00 ± 1.32
IC_{50}(µg/ml)	97.68 ± 1.08	91.53 ± 0.31

Table 1.2 : ABTS Radical Scavenging Activity of *Origanum vulgare*

concentration(µg/ml)	% scavenging (hydroalcholic extract)	% scavenging (std)
50	27.57 ± 0.43	31.01 ± 0.62
75	38.40 ± 0.33	47.13 ± 0.58
100	53.63 ± 0.34	63.65 ± 0.36
125	63.29 ± 0.77	71.75 ± 0.59
150	78.34 ± 0.61	80.14 ± 0.66
IC_{50}(µg/ml)	94.25 ± 0.84	82.22 ± 0.065

Std = BHT
Values are mean ± SEM (n=3)

Table 4.22: Lipid Per-oxidation Activity of *Origanum vulgare*

concentration(µg/ml)	% scavenging ((hydroalcholic extract)	% scavenging (std)
50	24.97 ± 0.97	35.50 ± 1.80
75	42.03 ± 0.72	46.01 ± 1.68
100	55.13 ± 0.07	53.53 ± 4.20
125	67.93 ± 1.07	65.59 ± 1.18
150	71.75 ± 0.30	75.70 ± 0.25
IC_{50}(µg/ml)	95.05 ± 0.12	85.12 ± 2.61

Std = Ascorbic acid
Values are mean ± SEM (n=3)

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