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EVALUATION OF HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF SEED EXTRACTS OF TUDRI SURKH (*CHEIRANTHUS CHEIRI*)

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ABSTRACT

Background: Hydrogen peroxide (H_2O_2) is not an inherently reactive compound but can be converted into highly reactive and deleterious hydroxyl radical (HO) that combines with nucleotides in deoxyribose nucleic acid (DNA) and cause strand breakage leading to carcinogenesis, mutagenesis and cytotoxicity. H_2O_2 scavenging activity of a compound serve as a significant indicator of its potential antioxidant activity. In Unani system of medicine seeds of Tudri surkh (*Cheiranthus* cheiri) are used either solitarily or in compound formulation for the treatment of various disease having oxidative stress as basis of their pathogenesis. **Aims and Objective**: To evaluate the H_2O_2 scavenging activity of Ethanolic and Hydroalcoholic seed extracts of Tudri surkh (*Cheiranthus cheiri*). **Material and Methods:**

Extracts were obtained by Soxhlet extraction with respective solvents. The hydrogen peroxide (H_2O_2) scavenging activity of phytoextracts was assessed by UV- Vis spectrophotometer using the method of Ruch et al., 1989. **Results:** The seed extracts were capable of scavenging H_2O_2 in a concentration dependent manner. At lower concentration the scavenging activity of ethanolic extract was better than hydroalcoholic extract that may be attributed to higher solubility of phenolic compounds in purely organic solvent as compared to the solvent containing a proportion of water. **Conclusion:** Ethanolic and hydroalcoholic extracts and hydroalcoholic extracts of seeds of Tudri surkh (*Cheiranthus cheiri*) exhibited H_2O_2 scavenging activity and possess good antioxidant property.

KEYWORDS: *Cheiranthus cheiri*, Tudri Surkh, Hydrogen peroxide, Ethanolic extract, Hydroalcoholic extract, Antioxidant.

INTRODUCTION

Free radicals are chemical entities characterised by high reactivity and are fundamental to any biochemical process being an essential part of aerobic metabolism. The free radicals generated from normal cellular metabolism include Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). The most common ROS are Hydrogen peroxide (H₂O₂), Superoxide anion (O_2^-), Peroxyl radicals (ROO⁻) and Reactive hydroxyl (OH⁻) radicals while RNS include Nitric oxide and Peroxynitrite anion (ONOO⁻).^[1] H₂O₂ is not an inherently reactive compound but can be converted into highly reactive and deleterious product. H₂O₂ may undergo transition-metal-dependent conversion into the extremely deleterious hydroxyl radical (HO). HO radicals combine with nucleotides in deoxyribose nucleic acid (DNA) and cause strand breakage leading to carcinogenesis, mutagenesis and cytotoxicity.

In all organisms, there exists a balance between rate of production and removal of free radicals known as oxidative balance. Any increase in the rate of production or decrease in the rate of removal of free radicals disrupts this balance thereby leading to Oxidative stress.^[2] Oxidative stress plays a major role in the pathogenesis of atherosclerosis, hypertension, diabetes mellitus, ischemic disease, malignancies and acute renal injury.^[3,4]

An antioxidant inhibits the process of oxidation and can be defined as "a compound capable of inhibiting oxygen mediated oxidation of diverse substances from simple molecule to polymer and complex bio-system". Antioxidants scavenge the free radicals by inhibiting lipid peroxidation and many other mechanisms thereby offering resistance against oxidative stress.^[1] Currently synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used. However, restriction on the synthetic antioxidants is being imposed because of their toxicity to liver and carcinogenicity.^[5] Traditional systems of medicine are being explored for rich and safe natural sources of antioxidants. Tudri surkh (*Cheiranthus cheiri*) is a herbal origin drug used either singly or in compound formulations for treatment of various ailments in Unani system of medicine.^[6,7] We conducted an in-vitro study with the objective of evaluation of antioxidant activity of seed extracts of Tudri surkh (*Cheiranthus cheiri*) to scientifically validate its use as antioxidant.

MATERIALS AND METHODS

Chemicals

Phosphate buffer, Gallic acid, Hydrogen peroxide were purchased from S.D fine chemicals Pvt. Ltd. Mumbai.

Plant material

Seeds of Tudri surkh (*Cheiranthus cheiri*) were procured from market and identified by a taxonomist at National institute of science communication and information resources (NISCAIR), New Delhi.

Preparation of seed extracts

100 grams of dried seeds were powdered and extracted exhaustively with hydroalcohol and ethanol by the method of Soxhletion. After removing from water bath and filtration respective solvents were recovered by distillation and were evaporated to dryness. The percentage yield of hydroalcoholic extract and ethanolic extract was 12.64% and 16.65% respectively.

Determination of H₂O₂ scavenging activity

The ability of seed extracts of Tudri surkh (*Cheiranthus cheiri*) to scavenge H_2O_2 was determined according to the method of Ruch et al., 1989. Seed extracts were prepared in different concentrations (100-1000 µg/ml) and mixed with H_2O_2 in phosphate buffer. Gallic acid was used as standard. After 10 minutes, the absorbance of reaction mixture was measured at 230nm against a blank solution containing phosphate buffer without H_2O_2 . For each concentration, a separate blank sample was used. The percentage H_2O_2 scavenging activity of plant extracts was calculated using the formula % of H_2O_2 Scavenging activity = (Abs _{control} – Abs _{sample}) / Abs _{control} × 100, where Abs _{control} = Absorbance of the control, Abs _{sample} = Absorbance of the extract/ standard.^[8]

Statistical analysis

All assays were carried out in triplicates and results are expressed as Mean ± Standard deviation. The IC50 values were calculated using MS Excel.

RESULTS AND DISCUSSION

Hydrogen peroxide (H_2O_2) is a weak oxidizing agent and can inactivate few enzymes directly, usually by oxidation of essential thiol (-SH). It can cross cell membranes rapidly and

inside the cell reacts with Fe^{2+} and Cu^{2+} ions to form HO radical which may be the origin of its toxic effects.^[9] It is biologically necessary for cells to control the amount of H₂O₂ and should not be allowed to accumulate. Flavonoids have antioxidant capability towards free radicals produced by cell metabolism and provide protection against the disease resulting as a consequent of oxidative stress. They control the oxidation at the cellular level by interfering with enzyme activity, chelating of redox active metals and by scavenging free radicals.^[10]

At a concentration of $100\mu g/ml$ ethanolic extract exhibited 58% scavenging activity on H₂O₂ while on the other hand standard and hydroalcoholic extract exhibited 18.64% and 39% scavenging activity on H₂O₂ respectively. 200 µg/ml of ethanolic extract showed 72% scavenging activity on H₂O₂ whereas hydroalcoholic extract and standard showed 54% and 41% respectively. At higher concentration of 1000µg/ml scavenging activity of both ethanolic and hydroalcoholic extract were closer to that of standard. Ethanolic and hydroalcoholic extracts exhibited 98% and 94.5% H₂O₂ activity while for standard it was 95.06%.(Fig 1). The IC₅₀ value of 278.45 µg/ml was found in standard. The IC₅₀ of 68.35 µg/ml was observed in ethanolic extract whereas IC₅₀ value of hydroalcoholic extract was 186.39 µg/ml.

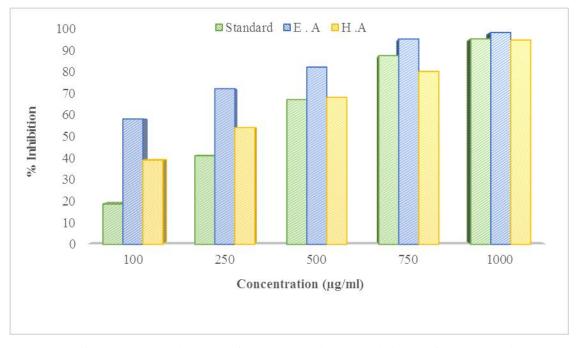


Figure 1: Graph showing H₂O₂ scavenging activity of Ethanolic extract, Hydroalcoholic extract and Standard at different concentrations. Tudri surkh (*Cheiranthus cheiri*) contains flavonoids like quercetin, erysmoside and alliside.^[11] Phenolics are the main antioxidant components of plant extracts and their total content is directly proportional to antioxidant activity of extracts.^[12] High concentrations of flavonoids in Tudri surkh (*Cheiranthus cheiri*) may be responsible for effective H₂O₂ scavenging activity of its extracts. The seed extracts were capable of scavenging H₂O₂ in a concentration dependent manner. At lower concentration the scavenging activity of ethanolic extract was better than hydroalcoholic extract that may be attributed to higher solubility of phenolic compounds in purely organic solvent as compared to the solvent containing a proportion of water. This is in conformity with an earlier study carried out by Quim Diem DO on freeze-dried *L. aromatica* extracts.^[13] At higher concentrations, the number of molecules with hydroxyl group will be proportionately higher in both the extracts and will, therefore, scavenge most of the H₂O₂ radicals present in the solution. It was observed that with the increase in the concentration above 750 µg/ml the scavenging activity of both ethanolic as well as hydro alcoholic extract was higher and comparable to the standard Gallic acid.

CONCLUSION

Ethanolic and hydroalcoholic extracts of seeds of Tudri surkh (*Cheiranthus cheiri*) exhibited H_2O_2 scavenging activity and possess good antioxidant property. Tudri surkh (*Cheiranthus cheiri*) can be investigated as safe and easily accessible source of natural antioxidants and can play a vital role in treating various diseases. Further studies need to be carried out to validate its antioxidant potential and ascertain the effectiveness of this drug as a good alternative to conventional antioxidants.

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