In vitro and in vivo antioxidant activity of Raphanus sativus leaves extract

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Abstract
Antioxidant are compounds of a natural or endogenous nature that either inhibit or interfere with toxic oxidants created and disable and there by prevent the spread of the chain reaction formed by these oxidants. The methanolic extract of Raphanus sativus leaves has been investigated for its in vivo and in vitro antioxidant activity. The in vitro antioxidant potency was evaluated by scavenging methods and it was observed that In DPPH Scavenging method Methanolic extract from Raphanus sativus leaves at concentration of (100μg/mL) had radical scavenging activity (IC\textsubscript{50}) of 117.30 µg/ml) in contrast to standardize ascorbic acid (100μg/mL) IC\textsubscript{50} (78.73 µg/ml). Whereas in Nitric oxide Scavenging method Methanolic extract from Raphanus sativus at concentration of (100μg/mL) had radical scavenging activity (IC\textsubscript{50}) of 213.04 µg/ml) in contrast to standardize ascorbic acid (100μg/mL) IC\textsubscript{50} (80.0 µg/ml). Apart from these actions, a significant in vivo antioxidant potential was also showed Raphanus sativus leaves methanolic extract exhibited a potent anti-oxidation action with respect to parameter like TBARS, GSH, Catalase and produced significant decrease in tissue MDA, lipid peroxidation & increase in Catalase and GSH. So, in the order of methanolic extract of Raphanus sativus leaves shows antioxidant activity.

Key words: Raphanus sativus, Antioxidant, Methanolic extract.

Introduction:
Raphanus sativus (Family: Brassicaceae) is the biological name for Radish (Diakon), It is a cultivated edible root crop. Aerial portions (leaves and stem) that are often discarded have been discovered to have significant antioxidant as well as radical scavenging ability, as determined by conventional antioxidant tests. \textsuperscript{56} Raphanus sativus is reported to have antimicrobial activity, anticancer, hepatoprotective, anti-diabetic, gastrointestinal and uterine tone modulatory, anti-ulcer and cardio-modulatory activities, treat piles, increases immunity and reduces fatigue and detoxifying agent. It's known that the physico-chemical, nutritive, antioxidant, as well as microbiological characteristics of leaves vary greatly. The most common free and bonded phenolic compounds in leaves and roots are pyrogallol and vanillic acid (correspondingly). Pyrogallol as well as vanillic acid are the two most commonly found phenolic compounds in leaves and roots.

Material and method:
Evaluation of in vitro Antioxidant activity:
Antioxidant potential are often assessed by examining the suppression of lipid peroxidation, hydrogen peroxide scrounging, and hydroxyl radical scavenging. The antioxidant activity of
various extracts was determined in vitro using a variety of different methods. Three techniques were used to determine the antioxidant working of a methanolic extract of *Raphanus sativus* leaves.

1. **DPPH Scavenging Activity**: The herbal blends' ability to scavenge free radicals has been evaluated using DPPH. In 95 percent methanol, a DPPH [1, 1-Diphenyl,2-picryl-hydrazyl] solution (0.004 percent w/v) was produced. To make the stock liquid mixture (1mg/ml), the methanolic extract of *Raphanus Sativus* was combined with the appropriate solvent. In test tubes, freshly produced DPPH solution (0.004 percent w/v) was put into it followed by successive serial dilution (25µg to 200µg) to achieve a final volume of 3 ml. After 10 minutes, the absorbance at 515 nm was measured using a spectrophotometer (Shimadzu UV – visible spectrophotometer). Ascorbic acid was utilized as a standard and dissolved in distilled water to create a stock solution with almost the same strength (1 mg/ml). Another control sample comprising the very same volume of extract as well as standards ascorbic acid has been produced. A specific solvent was used as a blank. The DPPH free radical scavenging activity was calculated.

2. **Nitric oxide scavenging activity**: Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated for 5 hours at 25°C with various concentrations of extracts (25-200µg/ml) dissolved in standard phosphate buffer (pH 7.4). After 5 hours, we withdrew 0.5ml of the incubated solution as well as diluted with 0.5ml containing Griess reagent. At 546nm, the absorbance of the generated chromophore was determined.

3. **Reducing Power Assay**: The reductive capacity of plant extracts was evaluated using the reaction mixture technique, which included varying concentrations of the herbal extract (25–200 µg/ml) and standard Ascorbic acid (2-16 µg/ml) in potassium ferricyanide [K3Fe (CN)6] (2.5 ml, 1% w/v), phosphate buffer (2.5 ml, 0.2 M, pH 6.6), and 1 ml distilled water was incubates at 50°C for 20 min. The reaction solution was in a fraction (2.5 ml) of 10% w/v trichloroacetic acid was mixed in the mixture, which would then be centrifuged at 1000rpm for 10 minutes. The top layer of fluid was combined with deionized water (2.5 ml) and FeCl3 (0.5 ml, 0.1 percent w/v), and the absorption at 700 nm was determined using a spectrophotometer. Increased absorption of the reaction medium showed that it has a higher reductive capability.

**Evaluation of in vivo Antioxidant activity:**

**Preparation of tissue homogenate:**
The animals were put under and then killed using diethyl-ether. After the organ was removed, it was placed in ice cold saltwater and then put on paper filters to dry before weighing. We used a tissue homogenizer in 0.15 M KCL to create 10% w/v tissue homogenate for TBARS, and we used 0.05 M phosphate buffer (pH 7.4) to prepare 10% w/v tissue homogenization for GSH and enzymes.

**Estimation of MDA**: A drop of homogenised tissue (made of 1ml of media) was collected from 10% tissue. They first added 0.5ml of 30% TCA and then added 0.5ml containing 0.08% TBA reagent. Next, the tubes were wrapped with insulating material and afterwards stored in a cold-water bath for 30 minutes. After this, they were put in a centrifuge at 3,000 RPM for 15 minutes. To measure the supernatant's absorbance, which may be used to assess the performance of absorbent products, absorbance was measured at 540 nm with a room temperature background. The ingredients included 1mL of pure water, 0.5ml of 30% TCA, and 0.5ml of 0.8% TBA.
Estimation of GSH: Mixing 2.5 ml of 0.02 M EDTA, 2 ml of 10% homogenate and 4 ml of distilled water, and 2 ml of the resultant mixture mixed with 1 ml of 50% trichloroacetic acid solution to the solution. Two milliliters of supernatant were transferred to four milliliters of Tris buffer (0.04 M, pH 8.9) mixed with 0.1ml of 0.01M DTNB solution, and shaken. After adding DTNB to be examined at 410 nm after 5 minutes with no homogenate against a reagent blank, the absorbance was measured. The micrograms of GSH per milligrams of protein were found.

Estimation of Catalase: Homogenization was carried out in a buffer solution (50 mM Potassium phosphate [pH 7.4]) with a 1:10 ratio of wet-to-dry mass. After being kept in a cooling centrifuge for 20 minutes, the sample was centrifuge at 10000 rpm at 4°C. To an assay vessel, which had been filled with 2.95 mL of a 19 mM/L solution of H2O2 in Potassium phosphate buffer, 50 µl of supernatant was added. H2O2 disappearance was tracked over 3 minutes using a 240 nm wavelength at one-minute intervals.

Result and discussion:
Antioxidant activity by DPPH method: The IC 50 Value of methanolic extract at different concentration of *Raphanus Sativus* was found to be 117.30 μg/ml in compression of standard ascorbic acid was found to be78.73µg/ml respectively. Fig. 1

![Fig. 1: DPPH scavenging activity of methanolic extract of *Raphanus Sativus*](image1.png)

Antioxidant activity by Nitric Oxide Scavenging method: The IC 50 Value of methanolic extract at different concentration of *Raphanus Sativus* was found to be 213.04µg/ml. in compression of standard ascorbic acid was found to be 80.0µg/ml respectively. Fig. 2

![Fig. 2: Nitric oxide scavenging activity for Methanolic extract of *Raphanus Sativus*](image2.png)
Antioxidant activity by Reducing Power Determination method: - the absorbance of methanolic extract at different concentration of *Raphanus Sativus* in compression of standard ascorbic acid shows potent antioxidant activity by reducing power determination method. Fig. 3

![Graph showing concentration response curve of Reducing Power determination for Methanolic extract of *Raphanus Sativus*](image)

**Fig. 3 Concentration response curve of Reducing Power determination for Methanolic extract of *Raphanus Sativus***

*In vivo Antioxidant of drugs:* Effect of extract of *Raphanus sativus* on liver *in vivo* antioxidant parameter tale 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Catalase (U/min/g)</th>
<th>GSH (μmol/mg)</th>
<th>MDA (nmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL CONTROL (Normal Saline)</td>
<td>83.2±3.6</td>
<td>5.3±0.33</td>
<td>206.7±7.1</td>
</tr>
<tr>
<td>NEGATIVE CONTROL (D- Galactosamine)</td>
<td>49.2±4.7***</td>
<td>2.3±0.26***</td>
<td>431.2±31.7***</td>
</tr>
<tr>
<td>POSITIVE CONTROL (Silymarin)</td>
<td>73.2±3.1**</td>
<td>4.4±0.26***</td>
<td>248.7±9.8***</td>
</tr>
<tr>
<td>RS Methanolic (300mg/kg)</td>
<td>53.5±2.8</td>
<td>3.9±0.13**</td>
<td>340.3±29.1</td>
</tr>
<tr>
<td>RS Methanolic (500mg/kg)</td>
<td>70.0±5.1*</td>
<td>4.4±0.93***</td>
<td>303.0±13.5*</td>
</tr>
</tbody>
</table>

![Graph showing Catalase level of *Raphanus sativus* extract](image)

**Fig. 4. Determination of Catalase level of *Raphanus sativus* extract**
Fig. 5: Determination of GSH level of *Raphanus sativus* extract

![GSH Graph]

Fig. 6: Determination of MDA level of *Raphanus sativus* extract

![MDA Graph]

**Statistical analysis:** Each value is represented as mean ±SEM, No. of animals (n) =6, ***P<0.01 Vs normal control
*P<0.05,**P<0.01 Vs cisplatin control, one way ANOVA followed by Dunett’s Test.

**Conclusion:** we tested the methanol extracts for antioxidant effects using DPPH and Nitric oxide methods. Since research has demonstrated that reactive oxygen species (ROS) are responsible for a variety of pathologies and various disorders. DPPH and Nitric oxide analysis are the best way to examine free radical scavenging ability and it is widely known to be used in testing antioxidant extracts. The standard ascorbic acid was compared to the IC 50 of *Raphanus sativus* methanolic extract in the DPPH scavenging technique and nitric oxide activity. *In vivo* antioxidant activity: The MDA level was significantly enhanced into albino Wister rats challenged by D-Galactosamine in contrast to animals from normal group. Administration of methanolic extract of *Raphanus sativus* leaves gave significant reduction in (P<0.01) in MDA value nearly close comparable to normal. GSH and Catalase were significantly altered in treatment with D-Galactosamine. Treatment with methanolic extract of *Raphanus sativus* the activities of glutathione as well as enzymatic proteineous antioxidants (P<0.01) came to near normal level.
References:


