ANTIOXIDANT ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACT OF PHYLLANTHUS NIRURI – INVITRO

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ABSTRACT

Medicinal plants are solving the health problems of world since decades and currently attracting the phenomenal interest at both the national and international level. Recently use of naturally occurring antioxidant for medicine or as a food material has been increased to avoid the side effects of synthetic antioxidants. Phyllanthus niruri (Linn) member of family Euphorbiaceae is one of the important medicinal plant, however less researched for its antioxidant activity and active ingredients. In this study antioxidant activity of ethanolic and aqueous extract of Phyllanthus niruri was analyzed with DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical and Hydrogen peroxide scavenging methods. Ascorbic acid, a standard antioxidant was used as control during the entire study period. The results indicate that ethanolic and aqueous extracts of Phyllanthus niruri are showing antioxidant activity with both the methods, however ethanolic extract showed high scavenging activity in comparison to aqueous extract with both the methods.

KEYWORDS: Antioxidant activity, DPPH, Hydrogen peroxide, Phyllanthus niruri.

INTRODUCTION

Antioxidants are molecules that prevent the oxidation of other molecules. Oxidation reaction produces free radicals and these may start chain reaction. When the chain reaction start in the cells may cause colossal damage or decease to the cells. In general, antioxidant breakdown
chains produced at the time of propagation process by giving an electron or a hydrogen atom to the free radicals and receiving surplus energy produced by the activated molecules (Lachman, 1986).

Oxidation reaction produces free radicals during metabolic processes are referred as reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS plays major role at lower concentration during pathogen attack etc. but produces oxidative and nitrosative stress if accumulated at higher concentration (Kovacic et al; 2001). Oxidative degradation of important biomolecules like nucleic acid, protein, lipid occurs during excess accumulation of ROS and leads to various disease like cancer, diabetes and cardiovascular disease (Dalle & Donne, 2006). Therefore toxic effect of RNS and ROS may be reduced by the use of antioxidant which is considered as boon for human health.

It has been recommended that plant sources like fruits, vegetables etc. are rich in substance called phytochemicals, considered as essential source of antioxidant in the diet and are capable enough to decrease the stress caused by ROS. The natural antioxidant may contain reducing agents, free-radical scavengers etc. (Ebadi, 2002). The antioxidants can neutralize the oxidation process by reacting with free radicals (Gupta, 2004). Currently health consciousness is increasing in mankind to find out natural antioxidants to restrict the side effect of synthetic antioxidant (Kumaran, 2007). Nowadays food industries are also using natural antioxidants over synthetic antioxidants (Govindarajan, 2003).

*Phyllanthus niruri* (Linn) is a member of family Euphorbiaceae considereded as a winter weed occurs across the hotter place in India. The *Phyllanthus* genus contains more than 600 species of shrubs, trees and annual or biennial herbs distributed throughout the tropical and subtropical areas (Paithankar, 2011). *Phyllanthus niruri* has vast benefits as an herbal medicine. The plant has been found to be hepatoprotective, antilithic, pain-relieving, antifungal, diuretic, antispasmodic, hypoglycemic, antiviral and anti-bacterial actions (Singh et al, 2016).

As per our literature survey it has been concluded that there is very less study has been conducted to study the antioxidant activity of areal part of *Phyllanthus niruri*. Therefore, the aim of the study was to evaluate the antioxidant activity of aqueous and ethanolic extract of areal part of *Phyllanthus niruri* by means of DPPH-scavenging and Hydrogen peroxide-scavenging methods.
MATERIALS AND METHODS
Areal parts (Dried leaves, flower and stem) of *Phyllanthus niruri* were collected locally from the campus of G.B Pant University of Agriculture and Technology, Pantnagar, Uttrakhand, India (29.500°N, 79.5167°E).

Ethanolic and Aqueous extraction preparation of *Phyllanthus niruri*.
Ethanolic and Aqueous extract preparation of *Phyllanthus niruri* were prepared by refluxing its 950g of areal parts along with 1.5 liter of related solvent.

Antioxidant Activity screening methods
Antioxidant activity of ethanolic and aqueous extract of *Phyllanthus niruri* was analyzed with DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical and Hydrogen peroxide scavenging methods.

**DPPH Radical Scavenging Method**
The free radical scavenging of both the ethanolic and aqueous extract was determined by DPPH method. In this method DPPH solution (0.004%), plant extracts and ascorbic acid (Standard) was prepared in methanol. Different concentration i.e. 10, 20, 40, 60, 80 and 100 μg/ml of plant extract and standard (ascorbic acid) were prepared and added in 0.5 ml of DPPH (0.004%) solution. DPPH and methanol in equal amount was kept as a control. Samples were incubated in the dark for 30 minutes and absorbance was recorded at 517 nm. All the samples were prepared in triplicate and assessed. Antioxidant activity decreases the absorbance of the DPPH radical and it is because of the scavenging of the radical by hydrogen donation. Color changes from purple to yellow and this is visually perceptible .The percentage inhibition activity was calculated using the formulae below.

\[
\text{DPPH Scavenging activity (\%)} = \left[\frac{A_0 - A_1}{A_0}\right] \times 100
\]

Where A0 is the absorbance of the control, and A1 is the absorbance of the extract/standard. The antioxidant activity of the extract was expressed as IC50. The IC50 value was referred as the concentration (in μg/ml) of extracts that inhibits the formation of DPPH radicals by 50% (Rachh et al; 2009).

**Hydrogen peroxide scavenging method**
Antioxidant of both the ethanolic and aqueous extract of *P.niruri* was determined on the basis of their hydrogen peroxide scavenging capability. A 20 mM Hydrogen Peroxide solution was prepared (pH 7.4) and various concentrations of plant extracts and standard ascorbic acid i.e
10, 20, 40, 60, 80 and 100 μg/ml were added to the hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230nm was evaluated after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. All the samples were prepared in triplicate and assessed. The percentage inhibition activity was calculated from \([ (A_0 – A_1)/A_0 ] \times 100\), where A0 is the absorbance of the control and A1 is the absorbance of extract/standard. The antioxidant activity of the extract was expressed as IC50. (Kumaran, 2007 and Srinivasan, 2007).

RESULT AND DISCUSSION
Antioxidant activity of *Phyllanthus niruri* was evaluated with ethanol and aqueous extract. Result of the experiment suggests antioxidant property by both the DPPH and Hydrogen peroxide scavenging methods but to varying extent (Table 1 and Table 2).

As per table no.1 and graph A of DPPH scavenging method, percentage of DPPH scavenging increases in both the extract (Ethanolic extract and Aqueous) along with control (Ascorbic acid) with the increase in concentration. However percentage of scavenging of ethanolic extract is greater than aqueous extract in all the concentrations. Ethanolic extract showed minimum of 10.6% scavenging of DPPH at 10 μg/ml concentration and maximum of 64.3% scavenging of DPPH at 100 μg/ml concentration whereas aqueous extract showed minimum of 8.8% scavenging of DPPH at 10 μg/ml concentration and maximum of 24.8% scavenging of DPPH at 100 μg/ml concentration. In Hydrogen peroxide scavenging method (Table No.2 graph B) Ethanolic extract showed minimum of 10% scavenging of Hydrogen peroxide at 10 μg/ml concentration and maximum of 88.5% scavenging of Hydrogen peroxide at 100 μg/ml whereas aqueous extract showed minimum of 7.1% scavenging of Hydrogen peroxide at 10 μg/ml concentration and maximum of 42.3% scavenging of Hydrogen peroxide at 100 μg/ml.

Table: 1 Antioxidant activity of *Phyllanthus niruri* using DPPH-scavenging method.

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>% Antioxidant Activity</th>
<th>Ascorbic Acid</th>
<th>Phyllanthus niruri</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ethanolic extract</td>
</tr>
<tr>
<td>10</td>
<td>9.2</td>
<td>10.6</td>
<td>8.8</td>
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<tr>
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<td>20.4</td>
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</tr>
<tr>
<td>100</td>
<td>94.4</td>
<td>64.3</td>
<td>24.8</td>
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Graph A: Graphical representation of Antioxidant activity of *Phyllanthus niruri* using DPPH-scavenging method.

Table: 2 Antioxidant activity of *Phyllanthus niruri* using Hydrogen peroxide method.

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>% Antioxidant Activity</th>
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<tr>
<td></td>
<td>Ascorbic Acid</td>
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Graph B: Graphical representation of Antioxidant activity of *Phyllanthus niruri* using Hydrogen peroxide method.
The ethanolic and aqueous extracts of *Phyllanthus niruri* exhibited antioxidant activity by inhibiting DPPH as well hydrogen peroxide may be due the presence of Flavonoids, Tannins, Phenol, Saponins, Phytosterols and Terpenoids like phytochemicals.

**CONCLUSION**

After comparing the results with standard it can be concluded that Ethanolic extract of Phyllanthus niruri showed greater antioxidant property in comparison to Aqueous extract by both the methods tested during experiment. Finally it can be concluded that Phyllanthus niruri is a potent antioxidant.

**ACKNOWLEDGEMENT**

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**REFERENCES**

