Antioxidant activity of acetone extract of *Naravelia zeylanica*

Dibinlal, Seethadevi, Sangeetha Sukumaran

Abstract

*Naravelia zeylanica* Linn., is a climbing tree. The current study was to integrate the antioxidant activity of the leaves of Acetone extract of *Naravelia zeylanica* Linn., belonging to the family Ranunculaceae, a popular drug in traditional medicine. Phytochemical investigation of Acetone extract of *Naravelia zeylanica* Linn., showed the presence of Sugar, Carbohydrates and Flavonoids. Jerzy W. Jaroszewski et al reported the presence of three simple benzamides, 3, 4-methylenedioxybenzamide, 4-methoxybenzamide and 4-hydroxy-3-methoxybenzamide. In the Acetone extract of *Naravelia zeylanica* Linn., shows Antioxidant activity by the method of Superoxide scavenging activity and Hydroxyl Radical Scavenging Activity. But it does not show any antioxidant activity in Nitric oxide Radical Scavenging activity.

Keywords: Acetone extract, *Naravelia zeylanica*, Antioxidant, Hydroxyl Radical Scavenging.

1. Introduction

Medicinal plants are the local heritage with global importance. The World is endowed with a rich wealth of medicinal plants. In the olden traditions local communities in every ecosystem from the Trans Himalayas down to the coastal plains have discovered the medicinal use of thousands of plants found locally in their ecosystem [1]. *Naravelia zeylanica* Linn., known as Vatakkoti in Malayalam. Belongs to the family Ranunculaceae. It is a scandent or climbing shrub with tuberous roots, wiry stem and strong tendrils; leaves 3-foliate, opposite, terminal leaflets modified into a 3-branched tendril, leaflets ovate-lanceolate, serrate or crenate, prominently nerved; flowers yellow, fragrant, in axillary and terminal panicles, sepals downy, petals linear-clavate, elongate; fruits aggregate of achenes, ending in twisted feathery tails. In 2005 Jerzy W. Jaroszewski et al reported the presence of three simple benzamides, 3, 4-methylenedioxybenzamide, 4-methoxybenzamide and 4-hydroxy-3-methoxybenzamide [2]. Hence the present investigation attempt to bring out antioxidant studies of the leaves of acetone extract of *Naravelia zeylanica* Linn [3, 4].

2. Taxonomy [8]

| Kingdom    | Plantae |
| Subkingdom | Viridiplanteae |
| Phylum     | Tracheophyta |
| Subphylum  | Euphyllyphytina |
| Class      | Magnoliopsida |
| Subclass   | Ranunculidae |
| Order      | Ranunculales |
| Family     | Ranunculaceae |
| Genus      | Naravelia |
| Botanical name | Naravelia zeylanica |

3. Material and Methods

3.1 Collection and Preparation of plant material

Fresh leaves of *Naravelia zeylanica* Linn was collected from Sreekandapuram, Taliparamba, Kannur during the month of March. The leaves were washed several times with water to remove soil and extraneous matters, the leaves were spread on trays and air dried for two weeks.
They were kept at room temperature so that all water were removed and dried for two weeks. The dried leaves were powdered and sieved through No.10 sieve and the coarse powder was collected for extraction.

### 3.2 Removal of Chlorophyll
The powdered leaf was packed in a filter paper in the form of a thimble and was pre extracted with petroleum ether (40 – 60 °C) in a Soxhlet extractor to remove chlorophyll, waxy matter etc. The marc was air dried and used for the preparation of acetone extract.

### 3.3 Preparation of Acetone Extract
The method employed for the extraction was continuous hot extraction. Defatted powdered drugs were packed in filter paper to form a thimble. The thimble was loaded in a Soxhlet extractor and extracted with acetone, till the extract coming out of the body of extractor was clear and colourless. When all the powder was extracted, the extracts were combined and 80% of the solvent was recovered using a distilling unit. The remaining solvent was removed by evaporation.

### 4. Evaluation of Antioxidant Activity
#### 4.1 Superoxide Scavenging Activity
##### i) Principle
Superoxide was generated by photo reduction of riboflavin and the amount was measured by the reduction of NBT. Scavenging activity was measured by inhibition of NBT reduction in the presence of test compound.

##### ii) Method
The reaction mixture contained 2650 μl of phosphate buffer, 100 μl NBT, 100 μl KCN, 50 μl riboflavin and different concentrations of ethanolic extract of the plant in a final volume of 3 ml. The tubes were illuminated by an incandescent lamp for 15 minutes. Optical density was measured at 530 nm before and after illumination. The percentage of inhibition of superoxide generation was evaluated by comparing the absorbance value of control and test.

\[
\text{Percentage inhibition} = \frac{C - T}{C} \times 100
\]

\( C \) = absorbance of control

\( T \) = absorbance of the test

#### 5. Hydroxyl Radical Scavenging Activity
##### i) Principle
Hydroxyl radical scavenging was measured by studying the competition between deoxyribose and the test compound for hydroxyl radical generated from the \( \text{Fe}^{3+}/\text{ascorbate/EDTA/H}_2\text{O}_2 \) system (Fenton reaction). Deoxyribose attacks the hydroxyl radical and eventually results in the formation of TBARS (thiobarbituric acid reactive substances), which is estimated spectrophotometrically.

##### ii) Method
The reaction mixture contained 500 μl buffer, 100 μl \( \text{H}_2\text{O}_2 \), 100 μl EDTA, 100 μl FeCl₃, 100 μl Ascorbic acid, 100 μl deoxy ribose and various concentration of extract in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37 °C. Took 800 μl from the reaction mixture; added 200 μl SDS, 1.5 ml acetic acid and 1.5 ml TBA and kept in boiling water bath for 1 hour. Cooled and added 1 ml of distilled 11000 water and 5 ml of pyridine: butanol mixture. Shaken well, Centrifuged and absorbance of the supernatant was taken at 530 nm.

### 6. Result

<table>
<thead>
<tr>
<th>S. No</th>
<th>Group</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>100 μg</td>
<td>11.11±0.020</td>
</tr>
<tr>
<td>3</td>
<td>200 μg</td>
<td>14.11±0.079</td>
</tr>
<tr>
<td>4</td>
<td>400 μg</td>
<td>18.06±0.020</td>
</tr>
<tr>
<td>5</td>
<td>600 μg</td>
<td>34.21±0.038</td>
</tr>
<tr>
<td>6</td>
<td>800 μg</td>
<td>51.56±0.33</td>
</tr>
<tr>
<td>7</td>
<td>1000 μg</td>
<td>55.74±0.040</td>
</tr>
</tbody>
</table>

± Standard deviation, *P>0.01 Vs Standard

### 7. Discussion
The present study reveals that the acetone extract of the leaves of *Naravelia zeylanica* Linn., has significant antioxidant activity by Superoxide Scavenging Activity method and hydroxyl radical scavenging activity method. Super oxide produced by the photo reduction of riboflavin was...
found to be inhibited by acetone extract of *Naravelia zeylanica* Linn. The concentration of the leaf extract needed for 50% inhibition of super oxide was found to be 800 µg.

The hydroxyl radical generated by Fe$^{3+}$/ascorbate/H$_2$O$_2$ system were inhibited by acetone extract of *Naravelia zeylanica* Linn). The concentration of the leaf extract needed for 50% inhibition of hydroxyl radical was found to be 800 µg. Hydroxyl radical is highly reactive and short-lived.

Super oxide anion is the first reaction product of O$_2$ and it is a short lived species, generated in situ in normal cells under pathological conditions. In addition the metabolism of xenobiotics and exposure to ionizing radiations also generates these species. The most important source of O$_2$ radical is oxidative enzymes among which are xanthine oxidase, NADPH/NADH oxidase, aldehyde oxidase and dihydroorotate dehydrogenase etc. The condition of cellular oxidative stress arises either from over production of O$_2$ or other oxidative free radicals and results in tissue injury. Superoxide generated from activated neutrophils stimulates mutagenesis *in vitro* and oxidative stress from chronic inflammation favor cancer development in many organs.

The hydroxyl radical is produced following reaction of O$_2$ and H$_2$O in the presence of metallic ions such as Fe$^{3+}$/Cu$^{2+}$. Lipid is very susceptible to OH radical attack and initiates lipid peroxidation. Also it induces conformational changes in DNA including strand breaks, base modification, damage to tumor suppressor gene and enhanced expression of proto-oncogenes.

### 8. Conclusion

In conclusion, the present study revealed that *Naravelia zeylanica* Linn is a promising plant for future studies towards drug development and also the antioxidant activity of *Naravelia zeylanica* Linn., which indicates the need for the evaluation of anticancer and anti-inflammatory activities.

### 9. Reference

6. John R. Dean Extraction Techniques in Analytical Sciences 2009, 128-130