Antibacterial activity, in vitro antioxidant activity and anthelmintic activity of methanolic extract of Plumbago zeylanica L. leaves

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ABSTRACT

The antibacterial activity, in vitro antioxidant activity and anthelmintic activity from methanolic extract of leaves of Plumbago zeylanica L. were investigated in the present study. Antibacterial activity of the extract was evaluated against four bacterial strains at 50 and 100 mg/ml concentrations. A marked inhibitory effect was observed against Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Salmonella typhi with MIC values 0.3125, 0.3125, 2.5 and 0.625 µg/ml respectively. In addition, reducing power assay, determination of total antioxidant activity and DPPH radical scavenging activity were performed to evaluate the in vitro antioxidant activity of the extract. The results indicated slightly higher antioxidant activity of the extract than α-tocopherol but lower activity than that of BHA (Butylated hydroxyanisole). At the concentration of 250 µg/ml, the extract showed noticeable DPPH radical scavenging activity. In DPPH radical scavenging activity, the IC 50 value was found to be 204.74 µg/ml. Anthelmintic activity of the extract was evaluated using adult Indian earth worms (Pheretima posthuma) and the results indicated a dose dependent increase in anthelmintic activity of the extract at 25, 50 and 100 mg/ml concentrations.

Key words: Plumbago zeylanica, Antibacterial assay, Antioxidant activity, Anthelmintic activity, DPPH radical scavenging.

INTRODUCTION

Natural antioxidants are extensively on use over the past years as according to biomedical view point free radicals are involved in many diseases. Most diseases like cardiovascular, cancer, osteoporosis, degenerative diseases etc. are shown to be linked with Reactive Oxygen Species (ROS) production and oxidative stress theory [1]. The free radicals mainly act by attacking the unsaturated fatty acid in the biomembranes which causes membrane lipid peroxidation, decrease in membrane fluidity, and reduction of enzyme receptor activity and damage to membrane protein which finally triggers the cell inactivation or death [2]. Antioxidants therefore could be used to block or reverse the harmful and pathologic action of the free radicals. The antioxidants generally scavenge the free radicals and detoxify the physiological system.

Recent studies and research in the field of free radical biology has claimed that foods and beverages containing antioxidants play a significant role in the prevention of cancer, cardiovascular and neurodegenerative diseases [3-9]. Of the two existing antioxidants viz natural and synthetic, natural antioxidants are preferred therapeutically and nutraceutically as synthetic antioxidants are suspected to have carcinogenic probability. As a result more and more antioxidants of natural origin are being investigated [9]. Since ancient days plants have been used for maintaining human health and improving quality of human life. Plants are serving the human community since time immemorial as food and beverage, as well as in cosmetics, dyes and medicines. On the basis of traditional folklore medicinal use in different communities of the world population, a large number of plants have been served as a valuable source of antibiotics, antioxidants and other therapeutic compounds. Sources of new pure compounds or leads in the process of drug discovery and developments are still from plants. In the recent years, there has been increasing focus that several plant derived polyphenolic compounds may possess antimicrobial, antioxidant, anticancer and apoptosis inducing properties [10]. Plants are considered as rich sources of antimicrobial agents. A wide range of plants and their parts are used for their medicinal properties by local communities and folkloric healers. Random screening as a tool in discovering new biologically active moieties has been most productive and successful in the area of antibiotics [8, 9].

Plumbago zeylanica L. is a semi-climbing shrub that grows throughout Asia and Africa. The whole plant and its roots have been used as a folk medicine for the treatment of rheumatic pain, dysmenorrhea, carbuncles, and contusion of the extremities, ulcers and elimination of intestinal parasites [10]. In traditional Indian medicine, Plumbago zeylanica L. has been assigned medicinal properties and is used in formulations for a number of ayurvedic compounds [11]. Plumbago zeylanica (Plumbaginaceae), locally known as Amera (in Amharic) is a shrub widely distributed in the West and Northwest parts of Ethiopia at 1500–2200m above sea level. It is also widely spread in tropical and subtropical regions of Asia, Australia and Africa [12]. In Ethiopia, it is traditionally used for the treatment of wound, eczema, scabies, leishmaniasis, leprosy and rheumatoid pain [13]. The roots of Plumbago species have been demonstrated to possess immunosuppressive and antitumor activities. Moreover, root extracts of Plumbago zeylanica are used by many of the population in South Africa as an oral treatment for complaints related to infections of the urinary tract.

The present study was designed to examine antimicrobial and antioxidant activities of methanolic extract of leaves of Plumbago zeylanica L. Preliminary phytochemical screening of the methanolic extract was also done along with the determination of total phenolic content. It was also of interest to find the anthelmintic activity of the extract against adult Indian earth worms to confirm the traditional folklore use of this plant in worm infestation.

MATERIALS AND METHODS

Plant material
Leaves of Plumbago zeylanica L. were collected from Mandi district of Himachal Pradesh (India) and authenticated by Dr. S. K. Sharma, Botanist, Research Institute in ISM, Joginder Nagar, Mandi, Himachal Pradesh. A voucher specimen herbarium was deposited in the Pharmacognosy department of Abhiliashi College of Pharmacy, Mandi, Himachal Pradesh for further reference.

Drugs and chemicals
Chemicals, such as Folin-Ciocalteau reagent, trichloroacetic acid (TCA), ethanol, methanol, ammonium thiocyanate, Dimethylsulphoxide (DMSO), Gallic acid, Tween 20, α-tocopherol, Butylated hydroxyanisole (BHA) were purchased from E. Merck (India) Limited, 1, 1 Diphenyl-2-picyl-hydrazyl (DPPH) was procured from Sigma, USA. Albendazole (BANDY, Mankind Pharma Ltd., New Delhi) was used as a standard anthelmintic drug. Petroleum ether, Tween 80 and all other chemicals and solvents used were of analytical grade available commercially (SRL, Mumbai, Himedia and E.Merck, India).
Preparation of extracts

The plant extracts were prepared by soaking hundred (100) grams of dry plant powder in 1 litre of 97% methanol for 4-5 days with intermittent shaking. At the end of extraction, it was passed through Whatman filter paper No.1 (Whatman Ltd., England). This methanolic filtrate was concentrated under reduced pressure on rotary evaporator at 40 °C and then stored at 4°C for further use.

Preliminary phytochemical screening

Identification of the chemical constituents were carried out on the powdered drug and on the methanolic extract using chemical methods according to the methodology proposed elsewhere [14].

Determination of total phenolic content

Total soluble phenolics in the leaf extract of Plumbago zeylanica L. were determined with Folin–Ciocalteau reagent according to the method using gallic acid as a standard phenolic compound. About 1.0 ml of extract solution containing 10 mg extract in a volumetric flask was diluted with 46 ml of distilled water. About 1.0 ml of Folin–Ciocalteau reagent was added and mixed thoroughly. Three minutes later 3.0 ml of 2% sodium carbonate was added and the mixture was allowed to stand for 3 h with intermittent shaking. The absorbance of the mixture was measured at 760 nm in a spectrophotometer (UV-1601 Shimadzu, Japan). The concentration of total phenols was expressed as mg/g of extract [15]. The concentration of total phenolic compounds in the extract was determined as gram of gallic acid equivalent using an equation obtained from the standard gallic acid graph:

\[ Y = 0.0024 \times + 0.0773, R^2 = 0.9504 \]

Where, \( Y \) was the absorbance and \( x \) was the concentration.

Antibacterial assay

Microorganisms

The microbial strains used for testing antibacterial activities included the following: Microorganisms.

Antibacterial assay

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Where, \( Y \) was the absorbance and \( x \) was the concentration.
Figure 1. Reducing power of a-tocopherol, BHA and *Plumbago zeylanica* L. methanolic extract

![Graph showing reducing power of a-tocopherol, BHA and *Plumbago zeylanica* L. methanolic extract.]

Figure 2. Total antioxidant activity a-tocopherol, BHA and *Plumbago zeylanica* L. methanolic extract at 250 µg/ml concentration

![Graph showing total antioxidant activity.]

Figure 3. DPPH radical scavenging activity a-tocopherol (Ascorbic acid), BHA and *Plumbago zeylanica* L. methanolic extract

![Graph showing DPPH radical scavenging activity.]

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Table 1. Antibacterial activity of methanolic extract of Plumbago zeylanica L.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Methanolic Extract 50 mg/mL</th>
<th>Zone of Inhibition (mm)</th>
<th>Methanolic Extract 100 mg/mL</th>
<th>Zone of Inhibition (mm)</th>
<th>Streptomycin 500 μg/mL</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>15</td>
<td>18</td>
<td>27</td>
<td></td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>17</td>
<td>22</td>
<td>30</td>
<td></td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>14</td>
<td>19</td>
<td>27</td>
<td></td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>11</td>
<td>19</td>
<td>26</td>
<td></td>
<td>100</td>
<td>40</td>
</tr>
</tbody>
</table>

Each zone of inhibition is an average of three independent determinations and the solvent (DMSO) did not show any inhibition.

Table 2. Minimum Inhibitory Concentration (MIC) of Plumbago zeylanica L. extract.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MICs of extract (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>0.3125</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0.3125</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>2.5</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>0.625</td>
</tr>
</tbody>
</table>

Table 3. Anthelmintic activity of Plumbago zeylanica L. methanolic extract.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Concentration (mg/mL)</th>
<th>Phorertia pathiana (Time taken for paralysis (P) in min. (Mean ± SD))</th>
<th>Time taken for death (D) in min. (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>1 % w/v Tween 80</td>
<td>No paralysis occurs (0 ± 0.4)</td>
<td>No death occurs</td>
</tr>
<tr>
<td>2</td>
<td>Piperazine citrate</td>
<td>15 mg/ml</td>
<td>13.800 ±2.000</td>
<td>13.500±1.761</td>
</tr>
<tr>
<td>3</td>
<td>Albenzole</td>
<td>20 mg/ml</td>
<td>11.533±4.165</td>
<td>11.83±0.752</td>
</tr>
<tr>
<td>4</td>
<td>Extract</td>
<td>25 mg/ml</td>
<td>40.000±2.191</td>
<td>45.000±0.894</td>
</tr>
<tr>
<td>5</td>
<td>Extract</td>
<td>50 mg/ml</td>
<td>33.33±1.751</td>
<td>37.500±1.049</td>
</tr>
<tr>
<td>6</td>
<td>Extract</td>
<td>100 mg/ml</td>
<td>26.83±1.69</td>
<td>33.000±1.265</td>
</tr>
</tbody>
</table>

In vitro antioxidant activity

Reducing power

Figure 1 shows the reductive capability of the extract compared to a-tocopherol and BHA. The measurement of the reducing ability, it was investigated the Fe²⁺-Fe³⁺ transformation in the presence of extract samples using the method of Oyaizu, (1986). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing power of the extract samples was increased with increasing concentrations. At all the studied concentrations, the plant extract showed slightly higher activity than a-tocopherol. Although reducing power of the extract samples was much lower than BHA. Reducing power of extract and standard compounds followed the order: a-tocopherol < extract < BHA.

Total antioxidant activity

Total antioxidant activity of the extract was determined by the thiocyanate method as described by Mitsuda et al., (1996). The plant extract exhibited effective and powerful antioxidant activity at 250 µg/mL concentration as shown in figure 2. The studied concentration of the extract (250µg/mL) showed higher antioxidant activity than that of 250 µg/mL a-tocopherol (26 %) but the antioxidant activity of the extract was lower than that of 250 µg/mL BHA (68.6%). Percentage inhibition of 250 µg/mL concentration of the extract in linoleic acid system was 49.6 %. On the other hand, percentage inhibition of 250 µg/mL concentrations of a-tocopherol and BHA (butylated hydroxyanisole) was found as 26% and 96.8% respectively. The antioxidant activity of the extract and standard compounds followed the order: a-tocopherol < extract < BHA.

Determination of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. Hence, DPPH is usually used as a substrate to evaluate antioxidant activity of antioxidants. Figure 3 shows a decrease in the concentration of DPPH radical due to the scavenging ability of the extract and standard compounds. BHA and a-tocopherol were used as standard radical scavengers. The scavenging effect of extract and standards on the DPPH radical decreased in the order of BHA > a-tocopherol > extract and were 87.15, 71.95 and 58.45 % at the concentration of 250 µg/mL, respectively. The IC₅₀ values were found to be 124.70, 160.31 and 204.74 µg/mL for BHA, a-tocopherol and extract respectively. From the results it was indicated that the extract have a noticeable effect on scavenging.
free radicals. Based upon the data obtained from this study, it is evident that the plant extract is a good free radical inhibitor or scavenger. It was also reported that oxidative stress, which occurs when free radical formation exceeds the body’s ability to protect itself, forms the biological basis of chronic condition [21]. As a promising antioxidant, the plant extract reacts with free radicals, which may limit free radical damage occurring in the human body. The equations obtained from the graph for the calculation of $IC_{50}$ values are as follows:

**BHA:**
\[
y = 0.3709x + 7.3467, \quad R^2 = 0.9525
\]

**α-Tocopherol:**
\[
y = 0.2893x + 5.622, \quad R^2 = 0.9216
\]

**Plumbago zeylanica L. extract:**
\[
y = 0.2433x + 0.1867, \quad R^2 = 0.9786
\]

**Anthelmintic activity**

From the results for anthelmintic activity the predominant effect of albendazole and piperazine citrate was to cause a flaccid paralysis of the worms. Albendazole by inhibiting micro tubule polymerization and thereby inducing immobilization produced paralysis and death of worms [20]. Data in table 3 and figure 4 reveals that all the three concentrations of extract of *Plumbago zeylanica L.* showed significant dose dependent anthelmintic property at 25, 50 and 100 mg/ml concentrations. Results clearly indicated that 100 mg/ml concentration of the extract has the highest potency as an anthelmintic (took least time to cause paralysis and death of worms) when compared to standard drug piperazine citrate and albendazole. The present study clearly indicated the traditional ethno medical claim of the plant *Plumbago zeylanica L.* as anthelmintic.

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**REFERENCES**


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