Phytochemical analysis, antioxidant and anti-inflammatory activity of leaves and bark of *Ceropegia rollae* Hemadri

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**ABSTRACT**

The purpose of the present study is to evaluate in vitro antioxidant activity and anti-inflammatory activity of methanolic extract of the leaves and the bark of the plant *Ceropegia rollae* Hemadri. The antioxidant activity of both leaves and bark extract was studied using FRAP and DPPH method. The in vitro anti-inflammatory activity and phytochemical characterization were carried using known protocols. The various phytochemical components such as total phenolics and flavonoids were determined. The plant *Ceropegia rollae* also contains tannins and ascorbic acid. This is related to the antioxidant activity of the plant *Ceropegia rollae* extract. The leaves show good antioxidant and anti-inflammatory activity as compared to the bark. These can be used as natural antioxidant and anti-inflammatory agents.

**Introduction**

Inflammation is an important adaptive rejoinder caused by noxious stimuli and conditions such as tissue injury to the animal. Mostly the oxidative stress and inflammatory establish a complex interaction. The endocellular state shows a key role in the activation and dysfunction of various immune cell (1-3). *Ceropegia rollae* Hemadri shows significant radical scavenging activity and also interact with various cell lines (4-6).

Free radicals are highly reactive species; they may have one or more than one unpaired electrons. Such as the superoxide, hydroxyl and peroxy radicals. They may easily attack the cell lines and convert the normal cell into pathogenic cell. These free radicals are responsible for the large nobler of the diseases in the human and the animals. Due to increase in the population and the industrialization, the free radicals are increased in the atmosphere. These free radicals affect the human life badly (6, 7). To overcome this, there is need of antioxidants. This antioxidant plays important role for the protection of the body against damage by the radicals. These free radicals are highly reactive. Oxygen radicals are called as ROS and produced by industrial pollution or they are formed by the biochemical process from various cells and the respiratory chain. These free radicals are the main factor for the lipid peroxidation. *Ceropegia rollae* has certain bioactive compounds, which have good antioxidant and anti-inflammation activities (8-10).

The plant *Ceropegia rollae* play an important role in inhibition of the various bacterial disorders and overcome the free radical activity and the inflammation. The phytochemicals present in the plant *Ceropegia rollae* are the tannins, terpenoids, steroids, phenols, cardiac glycosides, saponins, flavonoids, alkaloids and anthraquinones, determined by various protocols developed (11-13). They also give positive test for the flavonoids from the Mg-HCl along with the Zn-HCl (14, 15). The plant also gave Keller and Killani test for the glycosides and with the acetic anhydride and sulphuric acid for the ferric chloride as reagent test for the phenols (16, 17).

*Ceropegia rollae* is a tuberous herb, which is widespread to Western Ghats and Konkan region of Maharashtra, India. The Stem is pubescent, leaves are ovate, puberulous, cymes in subaxillary and terminal. Flowers are larger, with peduncle and having pedicals hirsute in nature. The flower is 2.3-3.5 cm long, tube with length 1.5-2.5 cm long, base slightly the inflated at all angles. Mostly the flower Petals are 8-16 x 2.8
mm, linear, oblong with straight, hairless. (18-21). Flowers are ciliate. They are inner erect, subclavate in nature. Flowering is in the month of August-September.

There are around 200 species of the *Ceropegia*, among which 58 species are endemic to the Western Ghats of Maharashtra, India (22).

In the present study, we have used the leaves and the bark of the plant. The bark and leaves show good anti-inflammatory and anti-oxidant activity.

**Materials and Methods**

**Chemicals and Reagents**

The chemicals used were of analytical grades. All chemicals were purchased from the S.D. Fine and Loba chemicals, Mumbai, India.

**Collection of Plant Ceropegia rollae**

The plant *Ceropegia rollae* was collected from the Rajapur region of the Maharashtra India, identified and authenticated by Dr. Arun Chandore, Abasaheb Marathe College, Rajapur, Tehsil of Ratnagiri District, Maharashtra, India. The specimen was kept in the Abasaheb Marathe College, Rajapur (Fig. 1-3).

**Experimental**

The plant material was dried in shade for 4 weeks, then it was dried in oven at 40 °C for six hours. Then dried plant material were crushed into fine powder. Both were kept in the glass bottles under inert atmosphere. Phytochemical testing carried out by given protocol and then other biological activity assays were carried using proper protocol.

**Determination of total phenolic content**

The total phenol present were determined by the known protocol. This protocol has the extract solution 0.3 ml with 1.5 ml of the reagent, 10% Folin-Ciocalteu’s then 7.5% of the sodium carbonate added. The mixture formed at dark for the 30 min. The absorbance was measured at 760 nm and can be done using the standard gallic acid curve.

**Determination of antioxidant activity of the plant Ceropegia rollae**

Antioxidant activity was inspected for the extract by using FRAP and DPPH assays. DPPH is well known standard. The FRAP reagent was prepared with 25 ml acetate buffer with 2.7 ml 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ) solution and 2.7 ml of the 20 mm of ferric chloride water solution. Each sample (150 µl) was mixed with 0.6 mg/ml methanol and 4.7 ml of the FRAP reagent and stirred for 5 min. The absorbance was measured at the 590 nm. The calibration curve method was used to study. The L-ascorbic acid was used as standard (22).

**DPPH radical assay**

DPPH assay was reported by Liyana-Pathirana and Shahidi in 2005. The DPPH was newly prepared by 27 mg of the DPPH dissolved in 100 ml of ethanol at -15 °C before use 160 µl of sample was allowed to react with 2840 µl of DPPH reagent and 190 µl of distilled
Phytochemical Analysis

The phytochemical analysis was carried for the leaves and bark extracts for saponins, terpenoids, flavonoids, tannins, and phenols using the fixed protocols. The FRAP and antioxidant activities of leaves and bark ethanol extracts were also studied (Table 6).

Results and Discussion

The present study reports the phytochemistry, antioxidant and the anti-inflammatory activity of the leaves and bark of the plant Ceropegia rollae. The study revealed the presence of the saponins, terpenoids, flavonoids, tannin, phenolin the in leaves and stem of the plant, using the fixed protocols. Mostly the ethanolic extract and water extract were used for the antioxidant activity by the FRAP and the DPPH assays, and showed excellent activity compared with standard drug. Ethanol extract showed the good antioxidant activity than the water extract compared with the standard. The result of the anti-inflammatory study showed the inhibition of albumin denaturation by the bark and leaves of the plant. Ethanol extract of the leaves and stem showed excellent anti-inflammatory activity.

Conclusion

We have studied the new species, Ceropegia rollae for phytochemistry, anti-inflammatory and the antioxidant activity. This shows the presence of the various phytochemicals in the leaves and root of the plant. The leaves show the excellent antioxidant and anti-inflammatory activity than the bark. We can use these compounds as the natural antioxidant and anti-inflammatory agent.
Table 6. The Phytochemical screening of the plant Ceropegia rollae

<table>
<thead>
<tr>
<th>Test</th>
<th>Leaves</th>
<th>Stem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannin</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Steroid</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Cardio glycosides</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Phenol</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Saponins</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

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Authors’ contributions

Concept and work plan was supervised by SN and GW. All the analytical experiments were performed by KP and GW. The manuscript writing and statistical calculation were done by GW. Necessary and final corrections were done by NM and VS. All authors have read and approved the final manuscript.

Conflict of interests

Authors do not have any conflict of interests to declare.

References


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