COMPARISON OF ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF WILD AND CULTIVATED VARIETY OF RAUWOLFA SERPENTINA

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INTRODUCTION

India has the oldest and richest diverse cultural tradition associated with the herbal medicinal plants for curing numerous ailments [1]. Almost, 80% of the world population is relying on herbal medicines for their health care benefits because plant-based treatments are safe, economic, accessible, reliable, and highly effective. Therefore, exponential growth in the overall demand for the herbal drugs and their products are reported globally. However, uncontrolled growth of the population and unplanned or excess use of plant species is making them endangered [2]. Approximately, 1/4th of all plant species in the world are at risk of being endangered or becoming extinct. Moreover, global warming and habitat destruction are also reasons of the disappearance of many plants. Some common plants which are become rare and endangered in the past 30 years due to habitat destruction are enlisted in Table 1.

In addition to this, there are some of the herbal plants which are used as substitute for the particular plants are Shatavari (Asparagus racemosus) is the substitution for Meda and Mahameda, Vidari (Pueraria tuberosa) substitution for Jivaka and Rasabhaka, Ashvagandha (Withania somnifera) Kakoli and Ksirakakoli, Guduchi (Tinospora cordifolia), or Gentaurae beenh substitution for Jivaka, Orchis spp. which includes Munjataka (Orchis latifolia Linn.), Yamsha Rochana (Bambusa arundinaceae) or Salvia haematodes substitution for Rasabhaka, Orchis mascula substitution for Meda, Gandharpasrani (Pseudustria foetida), Musali (Vetata Musali) (Asparagus adscendens) substitution for Mahameda, Talamuli (Krishna Musali) (Curculigo orchioides) substitution for Kakoli, and Chlorophytm arundinaceum substitution for Ksirakakoli.

Other than these, Rauwolfia serpentina is one of the endangered plants. Although it also has a huge demand for its alkaloids, as well as the raw drug in the international market. The requirement of dried roots of rauwolfia is around 20,000 ton/year [3] across the globe. It is commonly known as serpentina root, Indian snakeroot and belongs to the family Apocynaceae. It is a perennial glabrous herb or undershrub widely distributed in moist areas in subtropical Himalayas and plains from Punjab eastwards to Assam, Khasia Mountains, and Deccan Peninsula ascending to 1200 meters [4]. It has been used in India, since ancient time and reported in various texts of the indigenous system of medicine such as Ayurveda, Siddha, and Unani [5]. Dried roots of the plants are used for medicinal purpose and are mostly about 8–15 cm long and 0.5–2 cm in thickness subeylindrical curved and rarely branched. The outer surface is grayish yellow to brown with irregular longitudinal fissures. The fracture is short with slight odor and has bitter taste [6]. Rauwolfia serpentina is being used for the treatment of snakebites, mental illness, and blood pressure and it is highly reputed for hypertension, to treat painful affection of bowels, diarrhea, dysentery, cholera and colic, dyspepsia, epilepsy, giddiness, insomnia, and vitiated condition of kapha and vata [7,8]. It has been included in the Appendix II of “The Convention on International Trade in Endangered Species of Wild Fauna and Flora” (CITES) and in the negative list of exports plants by the Government of India (Notification no. 24 [RE-98]/1977-2002) [9].

It became endangered with extinction in India, due to its indiscriminate collection, limited cultivation, and huge industrial demand. Hence, cultivation of rauwolfia has been started to complete the demand. Therefore, the study was designed to evaluate and compare the in vitro antioxidant and antidiabetic activity of the wild and cultivated plant of rauwolfia.

ABSTRACT

**Objectives:** About 80% of world populations are still dependent on herbal plants. Rauwolfia is also one of the wonder drugs of India, which is used since ancient time. It contains a variety of compounds with antioxidant activity and other health benefits. A wild variety of rauwolfia is become endangered due to indiscriminate use. Hence, its cultivation and collection have been started to complete the demand of rauwolfia. Therefore, the study was designed to evaluate and compare the in vitro antioxidant and anti-diabetic activity of the wild and cultivated plant of rauwolfia.

**Methods:** The methanolic extract of wild and cultivated plant was subjected to the DPPH and alpha-amylase inhibition activity for antioxidant and anti-diabetic activity, respectively.

**Result:** The study revealed that the wild and cultivated variety of Rauwolfia serpentina does not have a significant difference in their anti-diabetic and antioxidant activities.

**Conclusion:** On the basis of the in-vitro studies, it can be concluded that cultivated variety of the plant can be used as a substitute for a wild variety of R. serpentina.

**Keywords:** Rauwolfia serpentina, Endangered plant, Antioxidant activity, Antidiabetic activity.
Experimental procedures for in vitro studies

Antioxidant activity
The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was performed with 700 µL of sample and MeOH (control) was added to the same volume of a methanolic solution of a 100 µM DPPH. Mixtures were shaken vigorously and left to stand in the dark at room temperature for 20 min, and then, the absorbance was read at 515 nm, using an ultraviolet spectrophotometer. Antioxidant activity was expressed as inhibition percentage (%) and calculated using the following equation.

\[
\text{Inhibition (I%) = } \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}} \times 100
\]

Table 2: DPPH assay

| S. No. | Concentration (µg/ml) | Inhibition (%) | Wide | Cultivated | Ascorbic acid |
|-------|-----------------------|----------------|------|------------|--------------|
| 1     | 0.1                   | 34.99          | 19.00| 45.56      |              |
| 2     | 0.2                   | 62.5           | 27.50| 52         |              |
| 3     | 0.3                   | 76.09          | 38.00| 62         |              |
| 4     | 0.4                   | 81.94          | 47.9 | 73.65      |              |
| 5     | 0.5                   | 94.64          | 55.00| 95.34      |              |

DPPH: 1,1-diphenyl-2-picrylhydrazyl

Table 3: α-amylase assay

| S. No. | Concentration (µg/ml) | Inhibition (%) | Wide | Cultivated | Acarbose |
|-------|-----------------------|----------------|------|------------|----------|
| 1     | 0.1                   | 16.68          | 13.70| 8.1        |          |
| 2     | 0.2                   | 21.79          | 18.73| 25.54      |          |
| 3     | 0.3                   | 24.32          | 20.11| 50.43      |          |
| 4     | 0.4                   | 26.30          | 25.43| 63.23      |          |
| 5     | 0.5                   | 58.43          | 51.80| 87.71      |          |

Experimental procedures for α-amylase assay were performed using dinitrosalicylic acid (DNSA) coloring agent. A total of 500 µL of R. serpentina plant extract and 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing α-amylase solution (0.5 mg/ml) and 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer were incubated for 10 min at 37°C. After incubation, 500 µL of sodium chloride was added to each tube at 5 s intervals, and then, 1 ml of DNA color reagent was added to stop the reaction. These test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. Finally, this reaction mixture was again diluted by adding 10 ml distilled water following which absorbance was measured at 540 nm.

\[
\text{Inhibition (I%) = } \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}} \times 100
\]

Table 1: Endangered plants in India

| S. No. | Region           | Botanical name                  | Common name          | Status* |
|-------|------------------|--------------------------------|----------------------|---------|
| 1     | Arunachal Pradesh| Amentotaxus assamica            | Assam catkin yew     | T       |
| 2     | Gujarat          | Polygala irregularis            | Milkwort             | R       |
| 3     | Gujarat          | Lotus corniculatus              | Bird’s foot          | R       |
| 4     | Kashmir          | Ceropogia odorata               | Jeemikanda           | E       |
| 5     | Hamimach Pradesh | Colchicum latense               | Gokhlicicum          | R, T    |
| 6     | Jammu            | Nymphea tetragona               | White Water-lily     | E       |
| 7     | Kashmir          | Nymphea tetragona               | White Water-lily     | T       |
| 8     | Karnataka        | Psilotum nudum                  | Fork fern            | R       |
| 9     | Karnataka        | Diosgyros celtica              | Ebony tree           | T       |
| 10    | Kerala           | Actinodaphne lawsonii           | Malayaram            | R       |
| 11    | Kerala           | Pterospernum reticulatum        | Malayaram            | R, E    |
| 12    | Madhya Pradesh   | Belosynopsis vivipara           | Jeemikanda           | E       |
| 13    | Rajasthan        | Ceropogia odorata               | Jeemikanda           | E       |
| 14    | Tamil Nadu       | Pterospernum reticulatum        | Malayaram            | T       |
| 15    | Tamil Nadu       | Acacia planifrons               | Umbrella tree        | R       |
| 16    | Tamil Nadu       | Abutilon indicum               | Ataliba              | R       |
| 17    | Tamil Nadu       | Chlorophytum malabaricum        | Malabar lily          | T       |

*(T: Threatened, R: Rare, E: Endangered)
There was a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme. The extract at a concentration 0.1, 0.2, 0.3, 0.4, and 1.0 µg/ml showed a percentage inhibition of 13.7, 18.73, 20.11, 25.43, and 51.80 for cultivated and 16.68, 21.79, 24.32, 26.30, and 58.43 for wild, respectively (Table 3). A comparative α-amylase % inhibition is plotted in Fig. 2 for wild, cultivated, and acarbose (a standard drug). The wild variety of the plant shows more inhibition as compared to the cultivated R. serpentina. The IC₅₀ calculated for wild variety is 0.83 µg/ml and cultivated is 0.96 µg/ml, respectively.

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