



Research Article

Pharmaceutical and nutraceuticals on three endemic species of *Ceropegia* Sp.

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Abstract: Plants are the richest resource of drugs of traditional systems of medicine and modern medicines. Medicinal plant species has its own nutrient composition besides pharmacologically important phytochemicals. Nutraceuticals is an emerging field of therapy. As we come to the end of this millennium, more and more people are getting health conscious and are looking at dietary substances for preventive or curative effects. The importance of minerals such as potassium, calcium, sodium etc. to human health is well known. The nutritional values and mineral composition of medicinal plants are being used as dietary supplements by humans. The nutritional compositions of selected *Ceropegia* species were found as good nutritive value. Nutritive compositions were analyzed and compared *in vitro* plant parts of *Ceropegia spiralis*, *Ceropegia candelabrum* and *Ceropegia juncea*. Sixty to seventy five days old *in vitro* plants were taken for biochemical studies of total proteins, total carbohydrates, sugar, starch and amino acid. Among the three samples analyzed, high protein sugar and aminoacids content was noticed in *Ceropegia juncea* and total carbohydrates and protein values high in *Ceropegia candelabrum*, *Ceropegia candelabrum* was found to be a rich source of iron (18.55 ± 0.11) followed by *Ceropegia juncea* (17.74 ± 0.25) and *Ceropegia spiralis* (17.56 ± 0.25). Nutraceuticals importance of these valuable plants may help in future for the production of new medicines and valuable food stuffs. The secondary metabolites present in them has a specific medicinal benefit other than a purely nutritional. These species are utilized for Indian system of medicine and scientists encourage them to rationalize it suiting the modern requirements of biotechnology.

Key words: Nutraceuticals; *In vitro*; *Ceropegia*; Species

Introduction

In modern medicine, plants continue to provide with us new chemical entities. Secondary metabolites are used as pharmaceutical, agro-chemicals, aromatics and food additives. Pharmacologists, microbiologists, biochemists, botanists and natural-product chemists all over the world are currently investigating medicinal herbs for phytochemical and lead compounds that could be developed for treatment of various diseases (Acharya *et al.*, 2011). In recent years, secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Erdo-grul, 2002; Krishnaraju *et al.*, 2005; Mahesh and Satish, 2008). The phytochemical investigation of raw drugs revealed a narrow spectrum of constituents in larger quantities traded in the market as the raw constituents (Uniyal *et al.*, 2006). Medicinal plant species has its own nutrient composition besides pharmacologically important phytochemicals. These nutrients are essential for the physiological functions of human body. Such nutrients and biochemical like carbohydrates, fats and proteins play an important role in satisfying human needs for energy and life processes.

The nutritive and medicinal values of the *Ceropegia* (Apocynaceae) species has been extensively used for the preparation nutritive tonic in Ayurvedic system. These *Ceropegia* species are placed under the categories of rare, endangered, vulnerable, extinct and threatened plants (Nayar and Sastry, 1987; Goyal and Bhadauria, 2006). Twenty-eight species of *Ceropegia* are endemic to the Peninsular India (Ansari, 1984; Ahmedulla and Nayar, 1986). Tissue culture techniques are the effective tools to conserve plants from extinction, homogenous production, maintain stable plant source produced them in large quantities and restore them to the natural habitats (Murch *et al.*, 2000a; Cambell *et al.*, 2003; Dave *et al.*, 2003; Bapat *et al.*, 2008; Sidhu, 2010). *Ceropegia* species are generally used for the preparation of medicine for the treatment of stomach and gastric disorders. Moreover, the tuberous roots of *Ceropegia spiralis* and *Ceropegia candelabrum* have great nutritive value. They are widely eaten by folks and other rural people.

Materials and Methods

Nutritional analysis

Sixty to seventy-five days old *in vitro* plants *Ceropegia juncea*, *Ceropegia spiralis* and *Ceropegia candelabrum* were taken for biochemical studies.

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*C. spiralis**C. juncea* tuber*C. candelabrum* tuberous root

Determination of total carbohydrate by anthrone method

Weigh 100 mg of the sample into a boiling tube. Hydrolyze by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCl and cool to room temperature. Neutralize it with solid sodium carbonate until the effervescence ceases. Make up the volume to 100 mL and centrifuge. Collect the supernatant and take 0.5 and 1 mL aliquots for analysis. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard. '0' serves as blank. Make up the volume to 1 mL in all the tubes including the sample tubes by adding distilled water. Then add 4 mL of anthrone reagent, heat for eight minutes in a boiling water bath. Cool rapidly and read the green to dark green color at 630 nm. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph calculate the amount of carbohydrate present in the sample tube.

Calculation

$$100 \text{ mg of the sample} = \frac{\text{mg of the glucose}}{\text{volume of test sample}} \times 100$$

Estimation of starch by Anthrone reagent

Homogenize 0.1-0.5 g of the sample in hot 80% ethanol to remove sugars. Centrifuge and retain the residue. Wash the residue repeatedly with hot 80% ethanol till the washings do not give color with anthrone reagent. Dry the residue well over a water bath. To the residue add 5.0 mL of water and 6.5 mL of 52% perchloric acid. Extract at 0°C for 20 min. Centrifuge and save the supernatant. Repeat the extraction using fresh perchloric acid. Centrifuge and pool the supernatants and make up to 100 mL. Pipette out 0.1 or 0.2 mL of the supernatant and make up the volume to 1 mL with water. Prepare the standards by taking 0.2, 0.4, 0.6,

0.8 and 1 mL of the working standard and make up the volume to 1 mL in each tube with water. Add 4 mL of anthrone reagent to each tube, heat for eight minutes in a boiling water bath. Cool rapidly and read the intensity of green to dark green color at 630 nm.

Calculation

Find out the glucose content in the sample using the standard graph. Multiply the value by a factor 0.9 to determine the starch content.

Estimation of sugar by anthrone method

Known amount of (100mg) *in vitro* plant sample cut into small bits and boiled in 20ml of distilled water in a boiling water-bath for 20 minutes and were needed for complete extraction of sugar in water. It was cooled and made up to known volume again with distilled water. 0.1 ml of the extract was taken and made up to 1ml with distilled water, 4ml of anthrone reagent was added and incubated in a boiling water-bath for 15 min. It was cooled and measured the absorbance of the blue color in a UV-Spectrophotometer at 620nm. The amount of total sugar present in the sample was calculated using the standard graph.

Extraction of protein from sample

Extraction is usually carried out with buffers used for the enzyme assay. Weigh 100mg of the sample and grind well with a pestle and mortar in 5-10ml of the buffer. Centrifuge and use the supernatant for protein estimation.

Estimation of protein

Pipette out 0.1 ml of the sample extract in test tubes. Make up the volume of 1ml in all the test tubes. A tube with 1ml of water serves as a blank. Add 5 ml of reagent (2% Sodium carbonate in 0.1N sodium hydroxide (Reagent A), 0.5% Copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in 1% Potassium sodium tartrate (Reagent B), Alkaline copper solution (Reagent C); Mix 50ml of A and 1ml of B prior to the use Reagent C to each tube including the blank. Mix well and allow standing for 10 min. Then add 0.5 ml of reagent D (Folin-Ciocalteu reagent), mix well and incubate at room temperature in the dark for 30 min. Blue color develops. Take the readings at 660nm in UV-Spectrophotometer. The absorbance was plotted against standard graph prepared with BSA and the amount of proteins were calculated and presented in the form of tables.

Amino acid quantification

Weigh 1gm of the *in vitro* plant sample and ground it in a mortar and pestle with 10ml of 80% ethanol. Filter through a fine cloth and centrifuged the filtrate at 500 rpm for 5 minutes. Discard the pellet and adjust the total volume of supernatant at 10 ml with 80% ethanol. This is the extract. Take 2ml of the extract and add 2ml of 0.1% ninhydrin reagent. Shake well and heat it in a boiling waterbath for 15

minutes until bluish purple color appears. Colour to room temperature and add 3ml of 50% ethanol. Prepare blank simultaneously. Optical density was read at 510nm. Extra polate the OD value and calculate the amount of amino acid present in the given plant tissue using standard graph.

Determination of ash content

5 gram of each powdered dried plant material (*in vitro* propagated plant material) was taken in the previously weighed porcelain crucible. The crucible was heated first over a low flame till all the material was completely charred, followed by heating in a Bunsen furnace for about 3 to 5 hours at 600°C. It was cooled in desiccators and weighed to ensure completion of ash formation. Further, it was heated again in the furnace for 30 minutes. Cooled and weighed. Repeat till the weight becomes constant.

Preparation of plant samples for mineral analysis

One gram of ash was taken in Kjeldhal flask, 20 ml of dilute HCL was added and digestion was carried out on a low flame initially for 10 to 15 min until frothing stops. The digestion at high temperature was carried out for 1 hour or till the contents of Kjeldhal flask become clear. Cooled the flask and transfer the content to 100 ml volumetric flask and adjusted the final volume to 100ml by adding distilled water. Use the solution for determination of mineral elements through the atomic absorption spectroscopy (AAS) and the flame photometry (FPM). Prepared the standard solution of each element drawn the calibration curves for each element using AAS/FPM.

Results

Table 1. Nutritional values of *Ceropegia juncea*, *Ceropegia spiralis* and *Ceropegia candelabrum* (100mg)

Plant name	Total proteins (Mean±SD)	Total carbohydrates (Mean±SD)	Sugar (Mean±SD)	Starch (Mean±SD)	Aminoacid (Mean±SD)
<i>Ceropegia juncea</i>	713.3±70.2	421.6±18.7	338.3±14.6	14.3±1.5	55.3±4.7
<i>Ceropegia spiralis</i>	681.6±12.5	344.3±4.0	288.7±10.1	14.0±1.4	44.4±3.1
<i>Ceropegia candelabrum</i>	383.6±3.2	461.6±2.8	272.6±6.8	15.3±2.0	31.4±0.9

Each value represents mean value ± SD of experiment carried out with three replicates.

On analysis of nutritional compositions, it was found that selected sampling plants contained varied protein contents. Protein content of *Ceropegia juncea* showed good protein content of 713.3 ± 70.2 mg/g followed by *Ceropegia spiralis* 681.6 ± 12.5 mg/g and *Ceropegia candelabrum* 383.6 ± 3.2 mg/g. On analysis of the results obtained, it was found that *Ceropegia candelabrum* contained the highest quantity of carbohydrates (mean ± SD = 461.6 ± 2.8 mg/g) followed by *Ceropegia juncea* (mean ± SD = 421.6 ± 18.7 mg/g) and *Ceropegia spiralis* (mean ± SD = 344.3 ± 4.0 mg/g).

In *Ceropegia juncea* the amount of sugar was 338.3 ± 14.6 mg/g followed by *Ceropegia spiralis* 288.7 ± 10.1mg/g and *Ceropegia candelabrum* 272.6 ± 6.8mg/g. Among the three samples analyzed, high starch content was noticed in *Ceropegia candelabrum* with mean ± SD value at 15.3 ± 2.0 mg/g, followed by *Ceropegia juncea* 14.3 ± 1.5 mg/g and *Ceropegia spiralis* 14.0 ± 1.4mg/g. On analysis, *Ceropegia juncea* showed high amino acid content of 55.3 ± 4.7 mg/g; followed by *Ceropegia spiralis* 44.4 ± 3.1 mg/g and *Ceropegia candelabrum* 31.4 ± 0.9mg/g.

Table 2. Macro and micronutrients of *Ceropegia juncea*, *Ceropegia spiralis* and *Ceropegia candelabrum* in ppm (1ppm=1mg)

Macro and micro nutrients	<i>Ceropegia spiralis</i>	<i>Ceropegia juncea</i>	<i>Ceropegia candelabrum</i>
Sodium	502.13±2.74	622.83±0.80	434.16±3.23
Potassium	325.86±0.90	431.8±1.11	321.13±0.61
Zinc	4.55±0.04	4.46±0.40	4.86±0.05
Iron	17.56±0.25	17.74±0.25	18.55±0.11
Copper	1.626±0.11	0.826±0.06	0.346±0.01
Cadmium	0.028±0.00	0.032±0.00	0.033±0.00
Nickel	0.056±0.00	0.046±0.00	0.043±0.00
Lead	0.076±0.00	0.040±0.00	-0.005±0.00

Each experiment carried out with three replicate. Values are represent mean ± SD.

The mineral elements contained in these plants are very rich. They are good for human consumption. The results of the mineral estimation of the *Ceropegia juncea*, *Ceropegia spiralis* and *Ceropegia candelabrum* are presented in table 2. Lead was totally absent in *Ceropegia candelabrum*. The species analyzed in this study contained remarkably high amount of sodium. *Ceropegia candelabrum* was found to be a rich source of iron (18.55±0.11) followed by *Ceropegia*

juncea (17.74±0.25) and *Ceropegia spiralis* (17.56±0.25). On analysis, it was also found that these plants were an excellent source of potassium ranging from 431.8±1.11 (*Ceropegia juncea*) to 325.86±0.90 (*Ceropegia spiralis*). Zinc content of the *Ceropegia* species was ranging from 4.86±0.05 (*Ceropegia candelabrum*) to 4.55±0.04 (*Ceropegia spiralis*) and 4.46±0.40 (*Ceropegia juncea*). These species showed to contained less than adequate level of

cadmium and nickel. The present study showed that the copper content was very less in *Ceropegia juncea* nearly 1.626 ± 0.11 in *Ceropegia spiralis*. Result analysis shows that all the three species are low in lead, cadmium and nickel constituents.

Discussion and Conclusion

The present study has clearly established the nutritional value of *Ceropegia spiralis*, *Ceropegia juncea* and *Ceropegia candelabrum* as food for humans (Table 1&2). Similar type of work was done by Deshmukh and Rathod, (2013). The tuber of *Ceropegia hirsute* and *Ceropegia bulbosa* and reported the presence of protein, carbohydrates, K, Na, P, Ca, Mg, Cu, Fe, Mn and Zn.

Carbohydrates are the important component widely found in plants, Starch is the most common digestible polysaccharide found in foods and therefore a major source of energy in our diets. Sugar, one of the major products of photosynthesis is synthesized in chloroplast (Horemans *et al.*, 1986). Sugar is used as a source of energy by crop plants and its level will decrease with the advancement of age. Among the sampling *Ceropegia* species maximum sugar content is present in *Ceropegia juncea*.

Plant food that provides more than 12% of its caloric value from protein is considered as a good source of protein. The plant *Ceropegia spiralis* and *Ceropegia juncea* is a good source of protein. According to Pamela *et al.* (2005) proteins from plant sources have lower quality but their combination with other sources of protein such as animal protein may result in adequate nutritional value.

Trace minerals are very important in a required amount, but excess or lower amount is harmful to the body. Similarly, mineral nutrition of *Ceropegia juncea* was reported by Karayil and Vivek (2014). The present study showed mineral estimation of the selected *Ceropegia* species in table 2. The obtained data reveals that the lead (Pb) constituents of 0.076 ± 0.00 in *Ceropegia spiralis* and 0.040 ± 0.00 in *Ceropegia juncea* are present. In the human body lead deficiency results in anemia, while excess lead (Pb) in the body causes brain damage (Stoker, 1976). Lead is a natural constituent of soil, water, vegetation, animal life and air. Significant sources of naturally occurring lead in air include silicate dust from soils and particles from volcanoes. Man is the leading contributor however, as he is manufacturing and using pesticides, combustion of coal, incineration of refuses and gasoline.

According to the Center for Disease Control and Prevention, iron deficiency is the most common nutritional deficiency in the United States. It is also the most common cause of anemia. Iron is very important in the formation of hemoglobin in red

blood cells and deficiency of iron leads to anaemia. The iron content of *Ceropegia candelabrum* (18.55 ± 0.11) followed by *Ceropegia juncea* (17.74 ± 0.25) and *Ceropegia spiralis* (17.56 ± 0.25). According to Bolt and Bruggenwert (1978) the recommended daily requirement of iron for man is 6-40mg/kg. Iron is required for hemoglobin formation (Alessandra and Robert, 2005). Certain trace elements such as copper, iron and manganese are to be included in a balanced diet. These micronutrients are sufficient in these three *Ceropegia* species. Some of them are micronutrient in plants and may not present in the right proportion. This will have adverse effect on both plants and human.

The present study shows that the cadmium content is 0.033 ± 0.00 of *Ceropegia candelabrum* 0.032 ± 0.00 in *Ceropegia juncea* and 0.028 ± 0.00 in *Ceropegia spiralis*. The concentrations of cadmium found in the present study were in accordance with the earlier reports. The normal limits of cadmium content in plants were between 0.2-0.8mg/Kg and toxic content of cadmium were defined as 5-30mg/Kg (Karayil *et al.*, 2011). Cadmium deficiency causes reduction in growth rate and excess cadmium results in hypertension and nephritis (Stoker, 1976).

Zinc is relatively non-toxic (Prasad, 1981). Zinc is necessary for the growth and multiplication of cells, skin integrity, bone metabolism, functioning of taste and eyesight (Thunus and Lejeune, 1994). Copper in human blood is principally distributed between the erythrocytes and the plasma. Total erythrocyte copper in normal humans is around 0.9-1.0 $\mu\text{g/ml}$ of packed red cells (Mason, 1979). The deficiency of copper has been associated with cardiac abnormalities in human and animal cause's anaemia and neutropenia (Mills *et al.*, 1976). Several micronutrients such as Cr, Mn, Fe, Cu, Zn etc in our diet in traces plays an important role in the metabolic processes. Their excess or deficiency may disturb normal biochemical functions of the body.

The present study shows that *Ceropegia* plants plays an important roles in the maintenance of health and curing of diseases. Therefore, an attempt has been made to show that these plants have the potential to be used as Nutraceuticals i.e. as food and medicine.

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