EFFECT OF COOKING ON PRIMARY METABOLITES OF  
PLECTRANTHUS AMBOINICUS (INDIAN BORAGE) 

Aabha Bhave¹ and Sumita Dasgupta² 
¹,²Biotechnology department of Bhagwan Mahavir college of science and technology, Surat, Gujarat, India 

Abstract 
Primary metabolites are vital for plant growth and many of them are pharmacologically active metabolites which can be use as antipsychotic drugs. Plectranthus amboinicus is an edible, nutritive plant and it is known to possess antimicrobial, antiepileptic and antioxidant properties. Cooking is a common practice to consume different leafy vegetables. Laboratory evaluations were made to assess the study of primary metabolites (Reducing sugar, protein, ascorbic acid) of P. amboinicus in raw and cooked samples. The result showed the difference in content of reducing sugar, protein, ascorbic acid in comparison of raw sample with cooked sample. 
Key words: Primary metabolites, reducing sugar, protein, ascorbic acid, P. amboinicus. 

I. INTRODUCTION 

Food which we eat provides us with energy and various other nutrients required for our healthy growth and development. Since ancient times medicinal plants are used as culinary adjuncts for treating or as a precautionary measure. Hippocrates said ‘Let food be thy medicine be thy foods’ [1]. India is a land of rich biodiversity. The total number of lower and higher plants in India is about 45,000 species [2]. Several culinary plants contain medicinal properties. P. amboinicus (Loureiro) Sprengel is a member of the family, Lamiaceae. or mint family. The paleotropical oil-rich genus, Plectranthus belongs to the subfamily Nepetoideae. It comprises about 300 species of annual or perennial herbs or subshrubs which are often succulents [3]. It is synonymous to Coleus aromaticus, Benth and is commonly known as Cuban oregano, Spanish thyme, Indian Borage, Mexican mint, etc. The herb has green, thick, succulent, heart shaped, leathery and juicy leaves with scalloped edges [4]. The raw leaves emanate an oregano-like flavor and odour when cut or crushed. Plectranthus amboinicus is an edible, nutritive plant [4] and it is known to possess antimicrobial, antiepileptic and antioxidant properties [5-7]. Several culinary usages of P. amboinicus have been reported in South America, Philippines, Indonesia, Africa, India and South East Asia [1]. In India P. amboinicus leaves are use for chutney and pakoda preparation. It is also used as condiment for sour soup in Vietnam, as principal flavouring in Cuban black bean soup and as salads in the Caribbean [1]. 

Primary metabolites are of prime importance and essentially required for growth of plants. Primary metabolites as essential nutrients are important components of crop plants for both consumers and producers [8]. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in of pharmaceutical compounds such as antipsychotic drugs [9-10]. The present work is to evaluate three important primary metabolites of raw and cooked leaf of plant P. amboinicus. 

II. MATERIALS AND METHODS

A. Collection of plant material-The young leaves of Plectranthus amboinicus were collected from Surat, Gujarat in month of December 2016, and authenticated by expert. The fresh leaves of plant
were thoroughly washed 2-3 times to remove adhering dust and impurities. The edible part were separated and blotted on filter paper, and use for raw and cooked extract of plant.

B. Sample preparation- Fresh green leafy vegetables were rinsed in water, dried on paper towel and the edible portions were separated from the inedible portion. The edible portions were chopped into almost equal small pieces or slices, mixed well and a portion (40 g) of the chopped vegetables was cooked by steaming in 200 ml of distilled water for 10 mins, while for raw extract a portion of plant were kept uncooked and used for analysis of reducing sugars [11-12], protein [13], ascorbic acid [14].

C. Estimation of Reducing Sugars

An aliquot from the extract prepared for the estimation of total soluble sugar was used for the estimation of total reducing sugars according to the Nelson-Somogyi method [11-12].

From the sample, a known volume of aliquot was pipetted out and was made up to 1.0 ml using distilled water. To this 1.0 ml of Somogyi’s copper reagent was added. The mixture was then placed in a bath of boiling water and heated for 20 minutes. After cooling under tap water 1.0 ml of Nelson’s arsenomolybdate reagent was added with immediate mixing till the effervescence ceased. The intensity of colour was measured after proper dilution at 540 nm using a Photochem Digital Colorimeter. D-Glucose was used as the standard.

D. Estimation of Proteins

Proteins Extraction:

Each of the plant parts were homogenized separately in 10% cold Tri Chloro Acetic acid TCA (10 mg: 5 ml) and were centrifuged at 5000 rpm for 10 minutes. Supernatant was discarded and pellets were saved. Pellets were again suspended in 5 ml of 10% cold TCA and re-centrifuged for 10 minutes. Supernatant was again discarded and the precipitate was dissolved in 10 ml of 0.1 N NaOH. 0.1 ml of this solution was used for protein estimation [15].

Quantitative estimation of Proteins:

In each of 1 ml extract, total protein content was estimated using the protocol of Lowry et al., 1951. A stock solution (1mg/ml) of bovine serum albumin was prepared in 1 N NaOH; five concentrations (0.2, 0.4, 0.6, 0.8 and 1ml) from the working standard solution were taken in series of test tubes. In another set of test tubes 0.1 ml and 0.2 ml of the sample extracts were taken and the volume was raised up to 1 ml in all the test tubes. To each test sample, 5ml of freshly prepared alkaline solution (prepared by mixing 50 ml of 2% Na₂CO₃ in 0.1 N NaOH and 1 ml of 0.5%CuSO₄. 5H₂O in 1% sodium potassium Tartrate) was added at room temperature and left undisturbed for a period of 10 min. Subsequently, to each of these mixture tubes 0.5 ml of Folin-Ciocaltcau reagent (diluted with equal volume of distilled water just before use) was rapidly added and incubated at room temperature (about 25°C) for 30 minutes until the blue colour developed. The spectronic colorimeter (Bausch and Lomb) was adjusted at wavelength of 750 nm and set at 100% transmittance using blank before taking the readings of the standard and the test samples respectively. Five replicates were examined in each case and their mean values were recorded. A regression curve was worked out of various concentrations of the standard solutions against their respective absorbances, which followed the Beer’s law [13].

E. Estimation of Ascorbic acid (vitamin C)

Sample preparation:

Five grams of sample were homegenized with 25 ml of metaphosphoric acid - acetic acid solution, and it was quantitatively transferred into a 50 ml volumetric flask and shaken gently to homegenize solution. Then it was dilute up to the mark by the metaphosphoric acid - acetic acid
solution. The obtained solution is filtered and centrifuged at 4000 rpm for 15 minutes, after which the supernatant solution is used for spectrophotometric determination (Perkin Elmer spectrophotometer Lambda 25) of vitamin C.

**Estimation of ascorbic acid Procedure:**

0.23 ml of 3% bromine water were added into 4 ml of centrifuged sample solution to oxidize the ascorbic acid to dehydroascorbic acid and after that 0.13 ml of 10 % thiourea to remove the excess of bromine. Then 1 ml of 2, 4-DNPH solution was added to form osazone. All standards, samples and blank solution were kept at 37 °C temperature for 3 hours in a thermostatic bath. After it all were cooled in ice bath for 30 minutes and treated with 5 ml chilled 85 % H₂SO₄, with constant stirring. As a result, a colored solution's absorbance was taken at 521 nm [14].

**III. RESULT AND DISCUSSION**

In the present investigation, *P. amboinicus* was evaluated quantitatively for the analysis of reducing sugar, total soluble protein, and ascorbic acid.

Glucose was used as a standard compound and reducing sugar were expressed as mg/g glucose equivalent (mg GE/gm) using the standard curve equation: \( y = 0.000x + 0.014 \), \( R² = 0.997 \), Where \( y \) is absorbance at 540 nm and \( x \) is reducing sugar content. (Fig. 1)

A reducing sugar is any sugar which can act as reducing agent because of free aldehyde and ketone group. All monosaccharaides, along with some disaccharides, oligosaccharaides and polysaccharaides are reducing sugar. The presence of reducing ends in the foods and the measurements of the concentration of reducing ends produced valuable information about the sample to be analyzed [16]. In present study, 0.84 mg GE/g reducing sugar was found in raw sample while 0.78 mg GE/g reducing sugar was found in cooked sample of *P. amboinicus*.

![Standard graph of glucose](image1.png)

**Figure 1. Standard graph of glucose**

![Standard graph of Bovine serum albumin (BSA)](image2.png)

**Figure 2. Standard graph of Bovine serum albumin (BSA)**
Bovine serum albumin (BSA) was used as a standard compound and protein were expressed as mg/g Bovine serum albumin equivalent (mg BSAE/gm) using the standard curve equation: $y = 0.000x + 0.028$, $R^2 = 0.998$, Where $y$ is absorbance at 750 nm and $x$ is protein content. (Fig. 2)

Proteins are the primary components of living things. The presence of higher protein level in the plant points towards their possible increase food value or that a protein base bioactive compound could also be isolated in future [17]. In present study, total levels of protein found to be 4 mg BSAE/g (0.4%) in raw sample, which was found to be quite similar as reported by khare et al., 2011 [4], while total levels of protein found to be 4.8 mg BSAE/g (0.48%) in cooked sample of *P. amboinicus*. Cooking caused an increase in the protein content of all the greens which might be due to greater moisture loss [18].

![Graph](image.png)

**Figure 3.Standard graph of Ascorbic acid**

Ascorbic acid was used as a standard compound and vitamin C were expressed as mg/g ascorbic acid equivalent (mg AAE/gm) using the standard curve equation: $y = 0.000x + 0.109$, $R^2 = 0.99$, Where $y$ is absorbance at 521 nm and $x$ is vitamin C content. (Fig. 3)

Ascorbic acid (vitamin C) is a familiar molecule because of its dietary significance, it is not only an important antioxidant, it also appears to link flowering time developmental senescence, programmed cell death and responses to pathogens through a complex signal transduction network [19-20]. In present study, total levels of ascorbic acid found to be 0.03 mg AAE/g (0.003%) in raw sample, which was found to be similar as reported by khare et al., 2011 [4], while total levels of ascorbic acid found to be 0.02 mg AAE/g (0.002%) in cooked sample of *P. amboinicus*. It is well established that vitamin C content are destroyed during cooking due to the fact that they are not stable at high temperature [21].

**IV. CONCLUSION**

In present study, it was found to be *P. amboinicus* is an outstanding source of sugar, protein and ascorbic acid. As cooking decreases the amount of sugar it can be appreciable for diabetes patients. As cooking increases amount of protein plant *P. amboinicus* can serve as a boon for curing ‘protein-malnutrition’ [15].As cooking decreases the amount of ascorbic acid particular attention to be paid to cooking procedure. It is recommended that addition of plant leaves to water only once it has reached boiling point to prevent loss of ascorbic acid [21]. *P. amboinicus* is easily available and easy to grow plant, can be used in a developing country like India where many people live their lives below poverty line and suffer from P.E.M. (Proteins Energy Malnutrition). *P. amboinicus* can be used to get the required nutritional requirement by consuming its leaves.
BIBLIOGRAPHY

[1] Wadikar, D.D. and Patki, P.E. 2016. Coleus aromaticus: a therapeutical herb with multiple potentials, J Food Sci Technol., 53(7): 2895-2901.
[2] Sathasivam, A. and Elangovan, K. 2011. Evaluation of phytochemical and antibacterial activity of Plectranthus amboinicus, International Journal of Research in Ayurveda and Pharmacy, 2(1): 292-294.
[3] Arumugam, G.; Swamy, M.K. And Sinniah, U.R. 2016. Plectranthus amboinicus (Lour.) Spreng: Botanical, Phytochemical, Pharmacological and Nutritional Significance, Molecules, 21(369): 1-26.
[4] Khare, R.S.; Banerjee, S. and Kundu, K. 2011. Coleus amboinicus hent: a nutritive medicinal plant of potential therapeutic value, International Journal of Pharma and Bio Sciences, 2(3): 488-500.
[5] Ragasa, C.Y.; Sangalang, V.; Pendon, Z. and Rideout, J.A. 1999. Antimicrobial flavones from Coleus amboinicus, Philippine Journal of Science, 128: 347-351.
[6] Pritima, R.A.; Selvaraj, R. and Pandian, R.S. 2008. Antimicrobial activity of Coleus aromaticus (Benth) against microbes of reproductive tract infections among women, African J Infect Diseases, 1: 18-24.
[7] Cano, J.H. and Volpato, G. 2004. Herbal mixtures in the traditional medicine of Eastern Cuba, Journal of Ethnopharmacol, 90: 293-316.
[8] Sato, F. and Matasui, K. 2012. 28 - Engineering the biosynthesis of low molecular weight metabolites for quality traits (essential nutrients, health-promoting phytochemicals, volatiles, and aroma compounds), plant biotechnology and agriculture, 443-461.
[9] Jayaraman, J. 1981. Laboratory Manual in Biochemistry. New Delhi: Wiley Eastern Limited, New Delhi.
[10]Bray, H.G. and Thrope, W.V. 1954. Analysis of phenolic compounds of interest in metabolism, Meth. Biochem. Anal., 1: 27-52.
[11]Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose, Journal of Biological Chemistry, 153(2): 375–380.
[12]Somogyi, M. 1952. Notes on sugar determination, Journal of Biological Chemistry, 195(1): 19–23.
[13]Lowery, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent, J.Biol.Chem., 193: 265-275.
[14]Mohammed, Q.Y.; Hamad, M.W. and Mohammed, K. 2009. Spectrophotometric Determination of Total Vitamin C in Some Fruits and Vegetables at Koya Area Kurdistan Region Iraq, Journal of Kirkuk University Scientific Studies, 4(2).
[15]Talreja, T. 2011. Biochemical estimation of three primary metabolites from medicinally important plant Moringa oleifera, International Journal of Pharmaceutical Sciences Review and Research, 7(2): 186-188.
[16]Journal of Kirkuk University Scientific Studies 2009, 4(2).
[17]Thomsen, S.; Handen, H.S. and Nyman, V. 1991. Ribosome inhibiting proteins from in vitro cultures of Phytolacea dodendra, Planta. Med., 57: 232-236.
[18]Kala, A. and Prakash, J. 2004. Nutrient Composition and Sensory Profile of Differently Cooked Green Leafy Vegetables, International journal of food properties, 7(3): 659-669.
[19]Nicholas 1996. The function and metabolism of ascorbic acid in plants, Annals of Botany, 78: 661- 669.
[20]Mapson, L. W. 1958. Metabolism of ascorbic acid system in plants. Part I. Function. Ann. Rev. Plant Physiol., 2: 119-150.
[21]Charlton, K.E.; Patrick, P.; Dowling, L. and Jensen, E. 2004. Ascorbic acid losses in vegetables associated with cook-chill food preparation, South African Journal of Clinical Nutrition, 17(2): 56-63.