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COMPARATIVE STUDIES ON PROXIMATE ANALYSIS AND AMINO ACID COMPOSITION OF LEAF AND FLOWER PROTEINS CONDUCTED ON MEDICINAL PLANT, *CATHARANTHUS ROSEUS*, AVAILABLE IN BANGLADESH

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ABSTRACT

Bangladesh is a rich store house of medicinal plants. *Catharanthus roseus* is administered as a cooling medicine. It is used for the treatment of diabetes, cancer, fever, malaria, cancer, throat infection and chest complaints. Taking all these things into consideration, it creates sufficient interest to carry out proximate analysis and amino acid compositions of leaf and flower protein of *Catharanthus roseus* from Bangladesh. The results of the proximate analysis showed that the leaves of *C. roseus* have high crude fiber (48.04%), carbohydrate (43.08%) and high protein content (8.08%) than flowers of *C. roseus*. The ash content for leaves is (15.21%) and for flowers is (8.37%). A total of 19 amino acid compositions were found for both leaves and flowers by amino acid analyzer of the dry powder of which eight are essential and nine are non-essential amino acids. Glycine (12.983 g/100 g), Arginine (3.612 g/100 g), Histidin (1.455 g/100 g), Methionine (1.201 g/100 g) and Glutamic acid (1.112 g/100 g) were found the most predominant amino acids in the analyzed sample of dry powder of leaves whereas Alanine (4.910 g/100 g protein) and Histidin (2.129 g/100 g protein) content were found the most predominant amino acids for the case of flowers of C. *roseus*. The non-essential amino acid

contained the highest for leaves (19.233 g/100 g protein) than the essential (6.248 g/100 g protein). And for flowers the non essential amino acid content contained the higher values (7.632 g/100 g protein) and essential amino acid content (4.769 g/100 g protein). The outcome of the study suggests that the leaf and flower of the plant have very good medicinal potentials, meet the standard requirements for drug formulations.

KEYWORDS: Catharanthus roseus, Proximate analysis, protein, Amino acids, essential amino acids, non-essential amino acids.

INTRODUCTION

Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grown in different parts of the country.[1] Today, according to world health organization (WHO) as many as 80% of the world's people depend on traditional medicine for their primary health care needs.[2] High plants are sources of drug which have made important contribution to the welfare and quality of life urban as well as rural communities especially in tropics and sub-tropics.[3] During the early years of human existence, many plants materials by instinct, intuition of trial and error were used to combat different aliments.[4]

Catharanthus roseus, as a medicinal plants (Common name - Periwinkle, Vinca; Bengali - Nayantara, Synonyms - Vinca rosea; Family - Apocyanaceae) popularly known as madagascar periwinkle is a potential source for anti-leukemic alkaloids. It is cultivated mainly for its alkaloids which are having anticancer activities.[5] It is an evergreen subshrub or harbeceous plant growing up to 1 m tall.[6] *Catharanthus roseus* is administered as a cooling medicine. It is used for the treatment of diabetes, fever, malaria, throat infection and chest complaints. It is also used for the regulation of menstrual cycles, and as a euphoriant.[7] The plant is an important source of indole alkaloids which are present in all plant parts. The physically important and antineoplastic alkaloids namely Vincristine and Vinblastine are mainly present in the leaves whereas antihypertensive alkaloids such as ajmalicine, serpentine and reserpine are reported to be present in the roots.[8] Vincristine and Vinblastine alkaloids are used in the treatment of various types of lymphoma and leukemia.[9,10] These Catharanthus alkaloids are also used for the treatment of both malignant and nonmalignant diseases and in platelet and platelet associated disorder.

The different parts of the plant, *C. roseus* are used for medicinal purposes for thousands of years in India, or subcontinent. It is one of the chief herbs for treating dermatitis, abscesses, eczema, psoriasis, sores, corns, ringworm, scabies, epilapsy, malaria, heart tonics and tumor. The plant has historically been used to treat a wide assortment of diseases. It was used as folk remedy for diabetes in Europe for centuries.[11] In India juice from the leaves was used to treat wasp sings. In Hawaii, the plant was boiled to make a poultice to stop bleeding. In China, it was used as an astringen, diuretic and cough remedy.[12] In central and South America, it was used as a homemade cold remedy to ease lung congestion and inflammation. Throughout the Caribbean, an extract from the flowers was used to make a solution to treat eye irritation and infections. It also had a reputation as magic plant. It was also observed that the leaves of it are used extensively in folk medicine for decreasing sugar level of blood and showed significant anti-hyperglycemic effect.[13-18]

Several studies have been carried out on the isolation of pharmacologically active compounds on leaves and flowers of *Catharanthus roseus* but a little work has been reported about proximate analysis of the leaves and flowers of the plant and amino acid composition of leaf and flower protein. Amino acids are the basic structural and functional units of proteins, thus amino acid have immense importance in the herbal medicine. Some proximate analysis works have been done previously on the leaves of this medicinal plant in some other countries like India, Pakistan, etc. But in Bangladesh no systematic proximate analysis as well as amino acid analysis on leaves and flowers of this plant was done so far. After all Bangladesh is not only geographically but also zoologically different from other countries. It is well known that physiological changes do occur in plants due to changes in geographical sites, climatic and environmental conditions which result in the production of nonidentical plant metabolites in the plant grown in different geographical regions. Keeping in mind the wide application of different plant parts of *C. roseus* in traditional medicine and ayurvedic preparation, proximate analysis of leaves and flowers and amino acid analysis of leaf and flower protein of the plant was carried out.

MATERIALS AND METHODS

Collection of plant material: Fully matured fresh leaves and flowers of *C. roseus* were collected from the gardens of Chemistry Department of Dhaka university , Bangladesh in June 2013 and identified by the taxonomist of Bangladesh national Herbarium, Dhaka, where a voucher specimen (No. = 39512) has been deposited. The leaves and flowers of *C.*

roseus were separately air dried. These dried samples of leaves and flowers were powdered using 20 mesh screen in Willey mill and then used for subsequent analyses.

Proximate Composition

Moisture and Dry matter contents: The Moisture content was determined by heating the samples in an electric oven at 105℃-110℃ until constant weight (6-10 hours) [19]. The percentage was calculated by

$$Moisture\ content\ (\%) = \frac{\text{Weight of moisture}}{\text{Weight of sample taken}} \times 100$$

Dry matter (%) = 100-moisture%

Ash Contents

Ash was determined by incineration of the moisture free samples at about 600℃ (about 6-12 hours) in a temperature controlled Muffle furnace until ash becomes almost white or grayish white in color.[19] The percentage of ash was calculated by

$$Ash\ content\ on\ dry\ weight\ basis\ (\%) = \frac{\text{Weight of ash}}{\text{Weight of sample taken}} \times 100$$

Acid insoluble Ash

Acid insoluble ash was determined by boiling the ash sample of leaves and flowers of *C. roseus* with 25 ml 3N HCl for 5 minutes and collecting the insoluble matter.[20] Then they were dried, ignited and weighed. The percentage from the ash taken was calculated by

$$Acid\ insoluble\ ash\% = Ash\ content\%\ (ODB) - \frac{(\text{Weight of ash taken} - \text{Weight of acid insoluble ash}) \times \text{ash content}\ (\%)}{\text{Weight of ash taken}}$$

Water Soluble Ash

Water soluble ash was determined by boiling the boiling the ash sample of leaves and flowers of *C. roseus* with 25ml distilled water for 5 minutes and the insoluble matter was thus collected.[20] Then they were dried, ignited at 450° C and weighed. The percentage from the ash taken was calculated by

$$Water\ soluble\ ash\% = \frac{(\text{Weight of ash taken} - \text{Wight of water in soluble ash}) \times \text{Ash Content}(\%)}{\text{Weight of ash taken}}$$

Nitrogen and Protein Content by Kjeldahl method

A known amount of fat free sample (after hot extraction with 40-60° C petroleum ether) of leaves and flowers of *C. roseus* was heated separately with conc. H_2SO_4 in presence of K_2SO_4 and $CuSO_4$ (98:2) in a long necked Kjedahl flask. When nitrogen present in the sample, it was converted into $(NH_4)_2SO_4$. The ammonium sulphate $(NH_4)_2SO_4$ thus obtained was boiled with excess of 60% NaOH solution (11-16 ml) to liberate NH_3 gas which was passed through in a conical flask containing 10 ml of 2% H_3BO_3 solution until 20-40 ml of distillate was collected (about 15 minutes). Excess NH_3 (in the receiving flask) were titrated against 0.1N sulphuric acid with mixed indicator methyl red and methylene blue (2:1). The end point was obtained at the reversion to the original greenish blue color.[21] A similar procedure was followed in the blank determination. The nitrogen and protein content of leaves and flowers of *C. roseus* is calculated according to the following formula,

$$Nitrogen\ (\%) = \frac{(\text{ml. standard acid} - \text{ml Blank}) \times \text{N of acid} \times 14 \times 100}{\text{Weight of sample taken in grams}}$$

Protein content (%) = Nitrogen content x 6.25

Crude Fiber

The moisture and fat free sample (after hot extraction with 40-60° C petroleum ether) (2-5 g) of leaves and flowers of *C. roseus* was digested with 200 ml boiling 0.255N H_2SO_4 (1.25% w/v) and 0.313N NaOH (1.25%w/v) solution. After digestion, it was dried at 110°C for 12 hours, ignite at 550°C in a Muffle furnace for 8 hours and recoded the loss of weight.[22] The percentage of crude fiber was calculated by

$$Crude\ Fibre\ (\%) = \frac{\text{Weight of sample after digesting with acid and alkali} - \text{Weight of ash}}{\text{Weight of dry sample taken}}$$

Carbohydrate Content

Carbohydrate content of leaves and flowers of *C. roseus* was estimated by subtracting the sum of the protein, fat, ash and crude fiber from the dry sample.

Carbohydrate (%) = 100- (Protein+Fat+Ash+Crude Fibre).

Essential oil Analysis by gas Chromatography coupled to Mass Spectrometry (GC-MS)

In the present study steam distillation method was used. In the process, definite amount of sample (fresh leaves (500 g) and flowers (300 g) of *C. roseus* separately) were taken in a distillation flask (Clevenger's apparatus). Then distilled water was added two third of its

volume to the flask. Then the flask was heated by electric heating mental for 4 hours. Volatile substances of leaves and flowers of *C. roseus* and generated steam in the flask were condensed by water condenser. The essential oil was lighter than water and so could be separated out. The steam distilled essential oil layer which was collected over water, was extracted and washed with analytical grade ether or chloroform. The ether extract of the oil was dried over anhydrous Na_2SO_4 and then filtered. It was collected in vial. The ether or chloroform was removed in vacuum condition. Thus the essential oil of fresh leaves and flowers of *C. roseus* was collected. The oil samples were stored in a refrigerator at -13° C until analyzed.

Calculation of Essential Oil Compositions

$$Relative\%\ of\ Individual\ Components = \frac{\text{Individual Area}}{\text{Total Area}} \times 100$$

Amino Acid Analysis

Sample Preparation for Amino Acid Analysis

The dry powder sample of leaves and flowers of *C. roseus* was (0.4 g for each case) refluxed with 6N HCl (50ml) at 110° C for 24 hours for protein hydrolysis. Then the solution was kept in an evaporating dish to evaporate HCl on water bath. The residue was again dissolved in 20ml 0.1N HCl and evaporation was repeated twice to remove excess of the acid. It was then filtered through Whatmann No.1 filter paper with 0.1N HCl in a volumetric flask which known to stock solution. Again, the stock solution was filtered through 0.45 µm syringe filter with pressure for amino acid analysis.[23] Then the stock solution and standard solution were run through Shimadzu Auto Amino Acid Analyzer (Shimadzu Corporation, Japan) with Fluorescent Detector. The amino acid content of leaves and flowers protein of *C. roseus* were calculated according to the following formula,

$$Amino\ acid\ Content\ (\%) = \frac{\text{Area of Sample}}{\text{Area of Standard}} \times \text{Concentration of Standard}$$

RESULTS AND DISCUSSIONS

Proximate composition of leaves and flowers of *C. roseus* were recorded and tabulated. The proximate composition of leaves and flowers of *C. roseus* is presented in Table 1.

Table: 1 Proximate composition of leaves of *C. roseus.*

Test parameters	Leaves	Flowers
	Percent (%) Composition	Percent (%) Composition
Moisture	76.20±0.23	43.20±0.02
Dry matter	23.80±0.23	56.80±0.20
Organic Content	84.79±0.12	91.63±0.30
Ash on drying	15.21±0.10	8.37±0.30
Acid Insoluble ash	0.32±0.02	0.21±0.05
Water soluble ash	2.21±0.01	1.01±0.02
Nitrogen (Kjeldahl method)	1.30±0.01	0.65±0.01
Protein	8.08±0.07	4.10±0.05
Crude fibre	2.10±0.50	1.04±0.01
Essential oil	0.05±0.09	0.07±0.09
Carbohydrate	43.08±0.30	31.23±0.05

Data are expressed as Mean ± SD (n=3)

The leaves of *C. roseus* have high crude fiber (48.04%), carbohydrate (43.08%) and high protein content (8.08%) than flowers. The ash content for leaves is 15.21% and for flowers is 8.37%. The present result found that the high value of ash in the case of leaves indicates high quality of mineral contents. The Moisture content in both the case was determined on the fresh weight basis whereas the organic content was calculated on the dry weight basis. Acid insoluble ash is an indication of silicate impurity and water soluble ash content indicates the higher soluble mineral contents in both cases. But in both cases leaves of *C. roseus* showed the higher values than flowers. Also in the current studies the acid insoluble ash was found very low and water soluble ash content was found in higher amount due to ash content in both cases. The protein content in the case of leaves of *C. roseus* was found 8.08% and in the case of flowers it was found 4.10%.

Crude fiber refers to the indigestible carbohydrate component that is present in plants. The name is derived from the fact that it has a naturally fibrous structure. Its primary purpose in plants is to form part of the structure in the cells but it is also useful for the human diet. In the intestinal tract, fiber resists being broken down by enzymes, although part of it may be metabolized by bacteria in the lower gut. Fiber is characterized by low or no nutritional value but because of its effect on the digestive system, it is thought to help with such problems in diabetes and high levels of blood cholesterol.[24] In the present study the crude fiber content in both the cases, i.e. for leaves (2.10%) and for flowers (1.04%) showed good values.

Like all living organisms, plants require energy in chemical form so they can grow and carry out basic life functions. Plants produce, store and burn carbohydrates in the form of sugar to provide themselves with energy.[25] Here in the present study showed the carbohydrate content in the case of leaves is 43.08% and in the case of flowers the content is 31.23%.

Amino acid composition of leaves and flowers of *C. roseus*

Every plant like any organism needs certain components for growth over and above soil, sun, rain and water. The basic component of living cell is protein with building block material amino acids. Proteins are formed by sequence of amino acids. The nutritive value of protein depends primarily on the capacity to satisfy the needs for nitrogen and essential amino acids. In our present study the amino acid profile of dry powder of leaves and flowers of *C. roseus* were analyzed by Shimadzu auto amino acid analyzer with an injection time of 35 minutes. A total of 19 amino acid compositions were identified for both leaves and flowers by amino acid analyzer of the dry powder. The amino acids were identified by comparing retention time with those obtained from standard amino acid retention time and calculate the percentage from their individual area. Table 2 and 3 present the amino acid profile of leaves and flowers of *C.roseus* of g/100 g protein. The percentage of amino acids groups are depicted in Fig 1 (for leaves & for flowers). The structures of amino acids present in the dry powder of leaves and flowers are shown in Fig 2 and 3. The results are calculated on the dry weight basis.

Glycine (12.98 g/100 g), Arginine (3.612 g/100 g), Histidin (1.455 g/100 g), Methionine (1.201 g/100 g) and Glutamic acid (1.112 g/100 g) were found the most predominant amino acids in the analyzed sample of dry powder of leaves of *C. roseus*. The non-essential amino acid contained the highest for leaves (19.233 g/100 g protein) than the essential (6.248 g/100 g protein).

Each amino acid has significant medicinal values. Glycine is used for treating schizophrenia, stroke, benign prostatic hyperplasia (BPH) and some rare inherited metabolic disorders. It is also used to protect kidneys from the harmful side effects of certain drugs used after organ transplantation as well as the liver from harmful effects of alcohol. Other uses include cancer prevention and memory enhancement. Some people apply glycine directly to the skin to treat leg ulcers and heal other wounds.[26]

Arginine is an amino acid that's so potent; scientists refer to it as the "Miracle Molecule." And for good reason, because our bodies convert Arginine into nitric oxide, a molecule that helps blood vessels relax and open wide to support blood flow. Enhance blood flow supports many important systems in our body i.e. overall Cardiovascular system, Immune system, Muscles, Bone & Skeletal system, male genitourinary system, blood vessel etc.[27]

Table 2: Amino acid compositions (g/100 g crude protein) for the dry powder of leaves of *C. roseus*

Serial No.	Name of Amino acids	Retention time	m/z	Area	Conc.(ppm)	Conc. g/100g
1	Arginine(ARG)	3.373	303.00	1787438	852.461	3.162
2	Hydroxyproline(HYP)	4.261	260.00	931920	0.000	0.0
3	Serine(SER)	-------	234.00	------	N.D.(W/B)	0.0
4	Glycine(GLY)	4.180	204.00	399517	232.887	12.983
5	Threonine(THR)	------	248.00	--------	N.D.(W/B)	0.0
6	Alanine(ALA)	6.034	218.00	134157761	44606.154	0.165
7	Methionine(MET)	7.983	278.00	23694082	323.813	1.201
8	Proline(PRO)	8.054	244.00	192593819	240.739	0.893
9	Lysine(LYS)	8.796	361.00	101155568	110.977	0.411
10	Histidin(HIS)	8.885	370.00	143830806	392.327	1.455
11	Valine(VAL)	9.188	246.00	116228945	169.994	0.630
12	Leucine(LEU)	10.827	260.00	542795865	233.171	0.865
13	Isoleucine(ILE)	11.293	260.00	475216316	247.451	0.918
14	Phenylalanine(PHE)	10.932	294.00	175539441	207.038	0.768
15	Cystenine(CYS)	12.788	497.00	40657373	70.033	0.259
16	Tyrosine(TYR)	12.523	396.00	438365	0.166	0.0006
17	Aspartic acid(ASP)	8.798	304.00	148643019	177.873	0.659
18	Glutamic acid(GLU)	9.344	318.00	91208024	299.999	1.112
19	Tryptophan(TRP)	9.837	333.00	202817	0.043	0.00015

Table 3: Amino acid compositions (g/100 g crude protein) for the dry powder of flowers of *C. roseus*.

Serial No.	Name of Amino acids	Retention time	m/z	Area	Conc.(ppm)	Conc. g/100g
1	Arginine(ARG)	3.356	303.00	2613808	164.071	0.609
2	Hydroxyproline(HYP)	4.067	260.00	269561	0.000	0.0
3	Serine(SER)	3.494	234.00	119304	37.401	0.139
4	Glycine(GLY)	4.199	204.00	470741	36.117	0.788
5	Threonine(THR)	4.353	248.00	254578	21.725	0.080
6	Alanine(ALA)	6.041	218.00	30239756	1323.343	4.910
7	Methionine(MET)	7.998	278.00	7231318	185.319	0.687
8	Proline(PRO)	8.051	244.00	81494913	191.021	0.708
9	Lysine(LYS)	8.799	361.00	21415568	44.058	0.163

10	Histidin(HIS)	8.882	370.00	112199711	573.899	2.129
11	Valine(VAL)	9.204	246.00	29646520	81.309	0.301
12	Leucine(LEU)	10.839	260.00	308258727	248.314	0.921
13	Isoleucine(ILE)	11.294	260.00	107726650	105.189	0.390
14	Phenylalanine(PHE)	10.944	294.00	95562301	211.354	0.784
15	Cystenine(CYS)	12.801	497.00	7890588	25.487	0.094
16	Tyrosine(TYR)	12.579	396.00	284247	0.202	0.0074
17	Aspartic acid(ASP)	8.807	304.00	23930407	53.699	0.199
18	Glutamic acid(GLU)	9.339	318.00	7798938	48.103	0.178
19	Tryptophan(TRP)	9.191	333.00	840328	0.337	0.0012

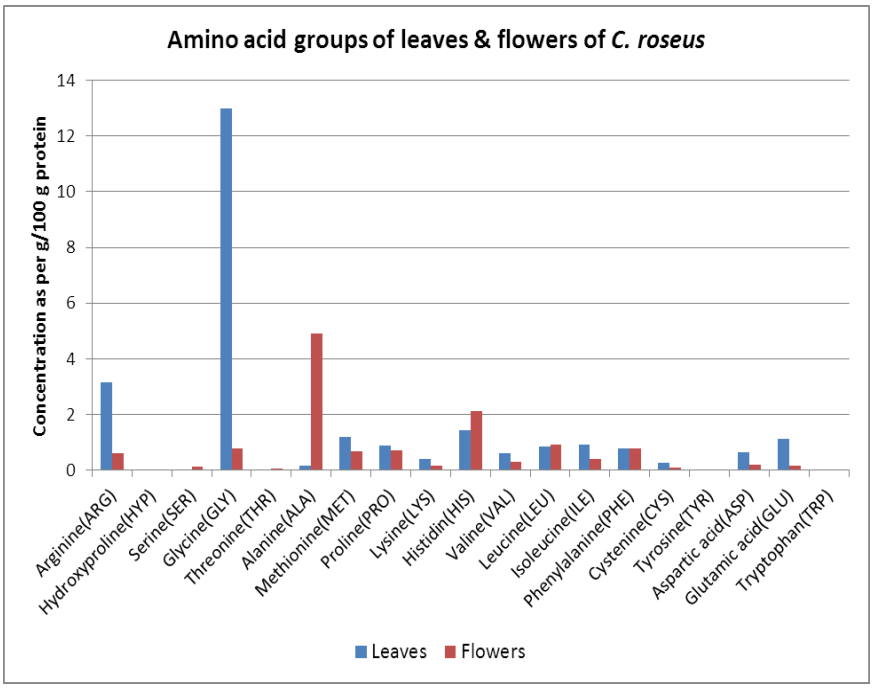
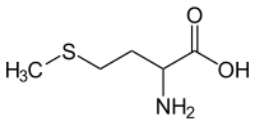


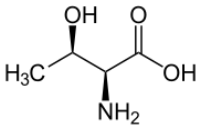
Fig: 1: Amino acid groups (g/100 g crude protein) of leaves & flowers of *C. roseus*

In the case of flowers of *C. roseus,* Alanine (4.910 g/100 g protein) and Histidin (2.129 g/100 g protein) were found the most predominant amino acids in the analyzed sample of dry powder. In flowers the non essential amino acids contained the higher value (7.632 g/100 g protein) and essential amino acids (4.769 g/100 g protein).

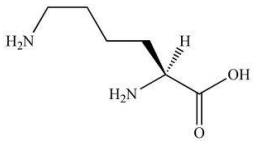
Alanine is a non-essential amino acid. Non-essential amino acids can be made by the body, so they don't have to be provided by food. Alanine is used for low blood sugar (hypoglycemia), diarrhea-related dehydration, liver disease, enlarged prostate (benign prostatic hypertrophy, BPH), fatigue, stress, and certain inherited disorders including glycogen storage disease and urea cycle disorders.[28] People uses histidin as medicine. Histidine is used for rheumatoid arthritis, allergic diseases, ulcers, and anemia caused by kidney failure or kidney dialysis.[29]



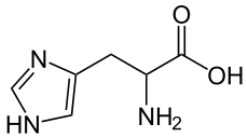
Methionine (MET)



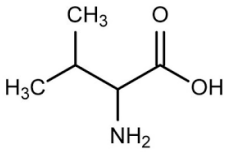
Threonine (THR)



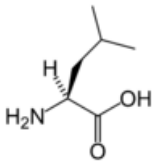
Lysine (LYS)



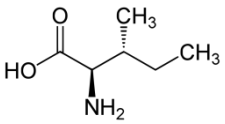
Histidin (HIS)



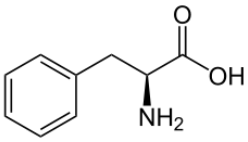
Valine (VAL)



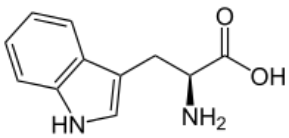
Leucine (LEU)



Isoleucine (ILE)

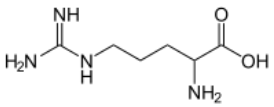


Phenylalanine(PHE)

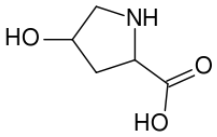


Tryptophan (TRP)

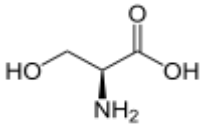
Fig: 2: Structure of Essential amino acids for leaves and flowers of *C. roseus*



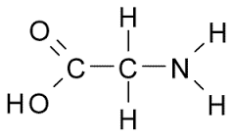
Arginine (ARG)



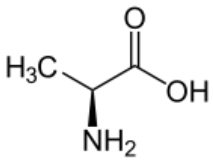
Hydroxyproline (HYP)



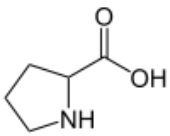
Serine (SER)



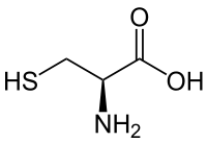
Glycin (GLY)



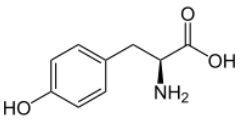
Alanine (ALA)



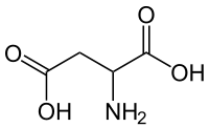
Proline (PRO)



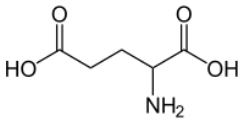
Cysteine (CYS)



Tyrosine (TYR)



Aspartic acid (ASP)



Glutamic acid (GLU)

Fig: 3: Structure of Non-Essential amino acids for leaves and flowers of *C. roseus*

CONCLUSION

The proximate analysis of leaves and flowers of the plant *Catharanthus roseus* showed that the leaves of the plant have high crude fiber (48.04%), carbohydrate (43.08%) than flowers. The ash content for leaves is (15.21%) and for the case of flowers the result found the content

(8.37%). The present result found that the high value of ash in leaves indicates high quantity of mineral contents. These results suggested that the plant parts could be an excellent source of investigated minerals and thus could help in maintaining normal physiological functions of human body. The protein content in the case of leaves of *C. roseus* was found 8.08% and in the case of flowers it was found as 4.10%. The storage of protein of different parts of the plant provides amino acids that are readily used for germination and seeding growth.[30] The proteins are considered for its essential amino acid profile due to the inability of the human body to synthesize them. Amino acids play central roles both as building block of proteins and as intermediates in metabolism. Leaves and flowers of *Cathranthus roseus* were found to be a rich source of various amino acids. Glycine, Arginine, Histidin, methionine and Glutamic acid were found the most predominant amino acids in the analyzed sample of dry powder of leaves of *C. roseus*. The non-essential amino acid contained the highest for leaves (19.233 g/100 g protein) than the essential (6.248 g/100 g protein). In flowers Alanine and Histidin were found the most predominant amino acids in the analyzed sample of dry powder. In case of flowers non essential amino acids contained the higher value (7.632 g/100 g protein) then the essential amino acids (4.769 g/100 g protein). The both plant parts under the study area are potential source of nutritive contents. These are retaining potential to synthesize the nutritive contents which play active role in metabolism.

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