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EVALUATION OF PRIMARY METABOLITES IN DIFFERENT ACCESSIONS OF *URGINEA INDICA*, KUNTH.

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Abstract: Quantitative analysis of primary metabolites such as Protein, Carbohydrate, Amino acids, Vitamins and lipids have been made in five different accessions of *Urginea indica* collected from various localities of Karnataka and Kerala Gubbi Ramanagar, Kerala(Trissur), Rangantitu island and foothills of Gopalaswamibetta. The bulb parts of the plant being medicinally important, it is essential to know its primary metabolites content in bulbs. The Primary Metabolites varied in these content in different accessions studied. Amino acids content varied from lowest (34.47µg/ml) Gubbi to highest in Kerala (52.38µg/ml). While protein content least found in Gubbi (17.04µg/ml) highest in kerala (85.82µg/ml). Carbohydrate least in Gubbi (42.65µg/ml) and highest in Gopalaswamibetta (91.43µg/ml) and lipids highest in Gubbi. In other 4 accessions ranged from (29.01µg/ml to 30.90µg/ml) almost similar lipid contents. Rangantitu showed highest Carbohydrate content in these bulbs while Kerala shows more of protein content and Gubbi stands first in their lipid Content whereas Vitamin C was found to be more in Gopalaswamibetta (80.55 µg/ml) followed by Rangantitu (74.5 µg/ml), kerala (54.35 µg/ml), Ramanagar(46.29 µg/ml) and Gubbi(38.23 µg/ml).

Keywords: *Urginea indica*, Amino acid, carbohydrate, lipid, Protein, Vitamin c

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INTRODUCTION

Plants form an important part of our everyday diet, and plant constituents and their nutritional value have been intensively studied for decades. Plants have been of immense important to human for production of metabolite which are the intermediates and product of metabolism. A substance essential to the metabolism of a particular organism or to a particular metabolic process is called metabolites. Metabolites have various functions, including fuel, structure, signaling, stimulatory and inhibitory effects on enzymes. A primary metabolite is a kind of metabolite that is directly involved in normal growth, development, and reproduction. It usually performs a physiological function in the organism (i.e. an intrinsic function). A primary metabolite is typically present in many organisms or cells.

Recently in India several scientists have reported the therapeutic importance of the chemical constituents of plants used in ancient Indian medical system. [1] *Urginea indica* commonly called as Indian squill belongs to the family Hyacinthaceae are bulbous plant which are excellent source of medicine with pharmaceutical and biocidal applications mainly as anticancer agent, expectorant, cardiac stimulant, hypertension, dyspepsia and arterio-sclerosis [2],[3],[4] in treatment of asthma, rheumatism, edema, dropsy, allergies[5] , gout and to treat various other ailments[6],[7] . *Urginea indica* being medicinally very important plant which has been by our work is gaining immense global importance in view of its multiple uses. The present study includes quantitative estimation of primary metabolites in *Urginea indica* and to know the variation among different accessions.

MATERIAL AND METHODS

The fresh bulbs of Different accession named Gubbi, Ramanagar, Kerala, Ranganatitu, and Gopalaswamibetta were collected and were used for estimation.

Fig-1 Gopalaswamibetta

Fig-A: Vegetative Phase

Fig-B: Reproductive Phase

Fig-2 Gubbi

Fig-A: Vegetative Phase

Fig-B: Reproductive Phase

Fig-3 Rangantitu

Fig-A: Vegetative Phase

Fig-B: Reproductive Phase

Fig-4 Ramanagar

Fig-A: Vegetative Phase

Fig-B: Reproductive Phase

Fig-5 Kerala

Fig-A: Vegetative Phase

Fig-B: Reproductive Phase

Estimation of Aminacids: Aminoacids are compounds containing one or more amino group and one or more carboxylic groups [8]. They are great biological importance since they play an important role as growth promoting factors and in Nitrogen metabolism. Some of the amino acids are supported to act as precursors of some secondary metabolites like alkaloids etc. They occur in plants both in free state and basic units of proteins and other metabolites [9], [10].

1 gram of material was weighed in every accession and extracted with 10 ml of Methanol. Centrifuged and supernatant was used as plant sample for extraction.

1 ml of plant material were taken from each accession and to that 1 ml of Ninhydrin reagent (0.8 grams stannous chloride in 500 ml of 0.2M citrate buffer and 20 grams of ninhydrin in 500ml of Methyl elusive or 2 methoxy ethanol) were added, kept it in water bath for 20 min. Then 5 ml of diluent solution were added to each sample and read the absorbency in 570nm. We used Leucine as standard amino acids. Standard was taken with concentration 0.2, 0.4, 0.6, 0.8 and 1 ml. Volume was made up to 1 ml. Then 1 ml of Nin hydrin was added and kept it for water bath for 20 min. Then 5 ml of diluents solution was added and measured the absorbance at 570nm.

Estimation of Protein

Proteins are present in the living world irrespective of the size of the organism, since they form the structural and functional basis of the cell. Under certain circumstances, it may become necessary to identify the presence of protein.

Protein was estimated by the method followed by Bradford [11]. Bradford method of protein estimation were carried out using coomassie brilliant blue G-250 staining reagent and Bovine serum Albumine as standard. Coomassie Brilliant Blue G-250 (100mg) was dissolved in 50 ml of 95% ethanol. To this solution 100 ml 85% of Phosphoric acid was added. Bovine serum Albumin standard solution of 100µg/ml was prepared. Dilutions were taken and using 5 ml of dye reagent absorbance were taken.

Estimation of Total Carbohydrate

Carbohydrates or sugars occupy a central position in plant metabolism. So that methods for their detection and estimation are very important to the plant scientist [12]

The basic unit of Carbohydrate are the monosaccharides which cannot be split by hydrolysis into simpler sugars. The Carbohydrate content can be measured by hydrolyzing the polysaccharides into simple sugars by Acid hydrolysis and estimating the resultant monosaccharides.

Total carbohydrate was estimated by the method followed by Anthrone[13]. Plant material was extracted with methanol and different concentration of plant material was taken and added 4 ml of Anthrone reagent. Boiled and cooled at room temperature. Colour was changed to Bluish green. Then the absorbance was measured at 630nm.

Estimation of Total Lipid

Lipids occurs in plant in bound form and also occur in in considerable amounts in seeds or fruits of a number of plants and provide such plants with a storage form of energy to use during germination.

Total lipid of plant material was estimated by Colorimetric method. 1 ml of plant material were taken and added 0.5 ml of concentrated sulfuric acid. Mixed properly and heated at 100 degree celceous for 10 minutes. Then cooled and added 5 ml of phosphovanillin reagent. Incubated at 37 degree celceous for 15 miutes and read the absorbance at 540nm [14].

Estimation of Ascorbic Acid or Vitamin C

Ascorbic acid otherwise known as vitamin C is an antiscorbutic. It is present in gooseberry, bittergourd etc. in high amounts. Generally, it is present in all fresh vegetables and fruits. It is a water soluble and heat labile vitamins.

Ascorbic acid was estimated by the spectrophotometric method [16]. Here ascorbic acid was taken as standard. 1 ml of plant sample was taken and in that 2.5ml of oxalic acid (0.05M) with EDTA(0.2M) was added. To that 0.5 ml of 5 % Sulphuric acid was and 2 ml of 5% Ammonium molybdate was added. Then 3 ml of distill water was added. Incubated for 15 min at 60◦celcious in boiling water bath. Then the intensity of blue colour was measured at 760nm.

RESULTS AND DISCUSSION

Variation in aminoaid quantity was observed in different accessions of *Urginea indica*

In which we can found kerala Aminoacid was found to be more about 52.38 µg/ml followed by Gopalaswamibetta (47.91 µg/ml), Ramanagar (38.97 µg/ml), Rangantitu (36.71 µg/ml) and Gubbi (34.47 µg/ml).

Extensive variation in nutrients and allelochemical composition of foliage has been documented within plants and subsequent experiment were conducted [17] to extend the range of Amino acid concentration present in the diet of Aphids. The improvements of the ninhydrin method make the method even more convenient, less expensive, and less time consuming for quantification of compounds containing amino group [18].

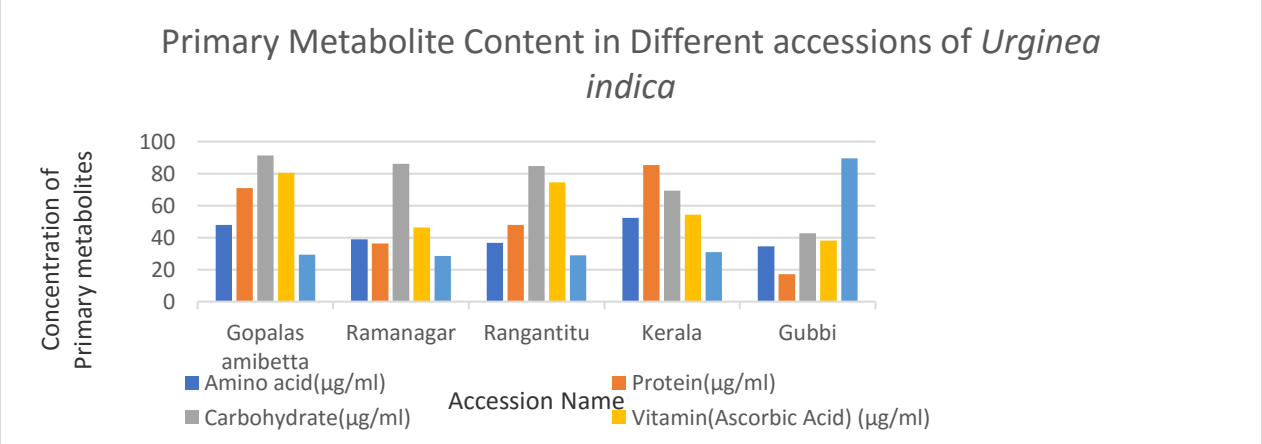
Protein content varied in different accessions of *Urginea indica*. Highest Protein content noticed in Kerala accession (85.42 µg/ml) followed by Gopalaswamibetta (40.93 µg/ml), Rangantitu (47.86 µg/ml), Ramanagar (36.30 µg/ml) and least in Gubbi (17.04 µg/ml) Nicholas J.Kruger observed variation in the response of proteins and effects of common reagent on the Bradford assay[19]. Linearization of the Bradford protein assay increases the sensitivity [20].

Carbohydrate content was found to be more in Gopalaswamibetta (91.43 µg/ml) followed by Ramanagar (86.16 µg/ml), Rangantitu (84.72 µg/ml), Kerala (69.36 µg/ml) and gubbi (42.65 µg/ml). varied carbohydrate content was also described by Sunita Kapila 2014 in three species of Marchantiaceae. An investigation of the reaction has therefore been carried out with a range of naturally occurring sugars and sugar mixtures, with a view to assessing the reliability of the anthrone reagent for the analysis of carbohydrates found in plant tissues [14]. As pointed out by Sattler & Zerban [21], it is very probable that the reaction between sugars and anthrone depends on dehydration and ring formation to form furfural or furfural derivatives which may then react with anthrone. The high specificity of the anthrone reaction for carbohydrate [22],[23] makes it particularly useful for the analysis of tissue extracts.

Lipid content was found to be more in Gubbi (89.47 µg/ml) while in other 4 accession it ranged between (28.63 µg/ml to 30.9 µg/ml) showing similarity in lipid content. whereas Vitamin C was found to be more in Gopalaswamibetta (80.55 µg/ml) followed by Rangantitu (74.5 µg/ml), Kerala (54.35 µg/ml), Ramanagar (46.29 µg/ml) and Gubbi (38.23 µg/ml).

To conclude Gopalaswamibetta accession of *Urginea indica* bulbs showed more of Carbohydrate while Kerala showed highest content of Protein and Amino acid among other accession. Lipid content was found to be more in Gubbi whereas Vitamin C content was more in Gopalaswamibetta accession.

Graph-1: Primary Metabolite contents in *Urginea indica* accessions



It is not clear whether the variations of primary metabolite contents in *Urginea indica* bulbs are biological significance or not but this variation may be due to ploidy difference among the accession or because of geographic distance between the 5 accessions.

Table-1: Primary Metabolite content in different accession of *Urginea indica*

Accession name	Amino acid (µg/ml)	Protein (µg/ml)	Carbohydrate (µg/ml)	Vitamin(Ascorbic Acid) (µg/ml)	Lipid (µg/ml)
Gopalaswamibetta	47.91±0.023	70.91±0.022	91.43±0.051	80.55±0.048	29.39±0.03
Ramanagar	38.97±0.028	36.30±0.031	86.16±0.029	46.29±0.025	28.63±0.024
Rangantitu	36.71±0.027	47.86±0.029	84.72±0.042	74.5±0.029	29.01±0.003
Kerala	52.38±0.045	85.42±0.045	69.36±0.023	54.35±0.033	30.90±0.013
Gubbi	34.47±0.041	17.04±0.047	42.65±0.033	38.23±0.046	89.47±0.005

Megastat was used to calculate the random error of variance for the concentration of each primary metabolites. Each value is represented as Mean±SD (n=4).

CONCLUSION

It is well known that primary metabolites add nutritional value required for plant growth and for animals that consume plants. The present study showed a significant variation in contents of primary metabolites such as Amino acid, Protein, Carbohydrate, Lipid and Vitamin C in five different accession of *Urginea indica* bulb. This variation may be because of geographic distance between the 5 accessions and also with local adaptation to environmental gradients and conditions. The obtained result may also contribute to the influence of ecosystem function on variation in phytochemicals in plants. The bulb of *Urgenia indica* can be recommended as a source of nutritional supplement. Further work on quantification of bioactive compounds and determination of their bioactivity can be developed as a functional ingredient in nutraceutical or pharmaceutical products

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