

## Asian Journal of Research in Biological and Pharmaceutical Sciences

Journal home page: [www.ajrbps.com](http://www.ajrbps.com)



### ANALYSIS ON THE FOOD VALUE AND CHLOROPHYLL CONTENTS OF VARIOUS PLANT SPECIES OF DEHRADUN

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#### ABSTRACT

*Triticum aestivum*, *Spinacia oleracea*, *Chromolaena odorata* plants have been analysed for the determination of food content viz, protein, glucose, ascorbic acid content and chlorophyll a, b and carotenoid. The concerned plants responded very well towards all the contents. Chlorophyll content was found highest in acetone and methanol extract of *Triticum aestivum*, *Spinacia oclera* followed by *Eupatorium odoratum*, but the diethyl ether extract of eupatorium posses more chlorophyll (a) content than that of *Triticum aestivum*, *Spinacia oclera*. The chlorophyll (b) content was found highest in the acetone extract of *Triticum aestivum* followed by diethyl ether extract of *Eupatorium odoratum*. The carotenoid content was found highest in all the acetone extracts of the concerned three plants followed by methanol extract, and fewer contents were found present in diethyl ether extracts of all plants. The glucose content was found highest in the acetone extracts followed by methanol extracts and lesser amount of carotenoids was found present in the diethyl ether extracts. Among the *Triticum aestivum*, *Spinacia oclera*, *Eupatorium odoratum*, the ascorbic acid was found highest in acetone extracts followed by methanol extracts and fewer in the diethyl ether extracts. The protein content was found highest in the diethyl ether extract *Spinacia oclera* followed by acetone extract and little amount was found in methanol extract of the concerned plants.

#### KEYWORDS

*Triticum aestivum*, *Spinacia oleracea*, *Chromolaena odorata* and *Eupatorium odoratum*.

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#### INTRODUCTION

Medicinal plants are well-known natural sources of remedies, used in the treatment of innumerable diseases since antiquity. Plants are invaluable sources of pharmaceutical products and Brazil has supplied an incredible array of medicinal plants that has drawn the attention of ethno pharmacologists around the world. It is estimated that there are 250,000 to 500,000 species of plants on Earth<sup>1</sup>. Phytochemical screenings are now seen as the first step towards the discovery of useful drugs that the

tropical rain forest has been identified as a potential source due to its diverse in flora. In order to understand these plants and for plants to reach rightful role in contributing to affordable healthcare, these plant must be assessed from a scientific viewpoint.

*Triticum aestivum* belonging to family poaceae is a green commonly found herb in India, although its nativity has been lost today. Wheat grass juice has chlorophyll that neutralizes infections, heals wounds, overcomes inflammations and gets rid of parasitic infections the three most important effects of wheat grass on the human body are: blood purification, liver detoxification and colon cleansing. This is because wheat grass juice is the richest source of vitamins A, B, C, E and K, calcium, potassium, iron, magnesium, sodium, sulphur and 17 forms of amino acids. Wheat, a cereal plant of the genus *Triticum* of the family Gramineae, is a major food and an important commodity in the world grain market. It is ranked third in total world production of cereal crops behind coarse grain and maize<sup>2</sup>. It was one of the first grains domesticated by humans<sup>3</sup>. These studies have indicated that whole wheat foods could reduce the risk of cancers<sup>4,5</sup> diabetes<sup>6,7</sup> and coronary heart disease<sup>8,9</sup>.

Spinach (*Spinacia oleracea* L.) is a cool season annual herb that belongs to the goosefoot family (Chenopodiaceae). It is low in calories and contains appreciable amounts of vitamins A and C and minerals especially iron. An earlier study on the edible portion (87%) of spinach records (in %): moisture, 94.3; protein, 2.2; fat, 0.7; fiber, 0.6; mineral matter, 1.7; carbohydrate, 2.9; and oxalic acid, 658 (mg/100g). Mineral composition includes (mg/100g): calcium, 73; magnesium, 84; potassium, 206; iron, 10.9; phosphorus, 21; sodium, 58.5; copper, 0.01; sulphur, 30; nickel, 0.42; manganese, 9.61; molybdenum, 0.08; zinc, 13.53; and strontium, 0.077. Spinach is a good source of the vitamin B complex, ascorbic acid, vitamin A and carotene. It is also a natural source of vitamin K. Studies have shown that spinach<sup>10</sup> is a short duration vegetable crop, its growth; yield and quality are largely influenced by the application of fertilizers. It

requires proper and sufficient N and K for regular growth. The vegetables are very good sources of vitamins such as vitamin A, vitamin B, vitamin C, riboflavin, and folate, and dietary minerals including calcium, iron, magnesium, phosphorus, potassium, zinc, copper, and manganese Ansari<sup>11</sup>.

*Chromolaena odorata* (*Eupatorium odoratum*) is the member of the family Asteraceae, (Compositae). It is a native of Central and South America which has spread throughout the tropical and subtropical areas of the world. Traditionally, fresh leaves or a decoction of *C. odorata* have been used throughout Vietnam for many years as well as in other tropical countries for the treatment of leech bite, soft tissue wounds, burn wounds, skin infection and Dento alveolitis. An aqueous decoction of the roots is used as an antipyretic and analgesic remedy, and a leaf extract with salt is used as a gargle for sore throats and colds. *Eupatorium odoratum* weed manures assist to compensate the deficiency of organic matter content along with nutrients in the soil and acts as ideal substitute against inorganic fertilizers. Organic manures are not only sources of major nutrients but they also provide other micronutrients and plant growth-promoting molecules, which together lead to good crop yields showed by Mader *et al.*<sup>12</sup>. The reason for increased nutrients could be attributed to solubilisation effect of plant nutrients by the addition of organic manures leading to increased uptake of NPK Sendur kumaran *et al.*<sup>13</sup>. The significance of organic manuring in sustainable agriculture is well established by Gaur *et al.*<sup>14</sup> and Subbarao *et al.*<sup>15</sup>.

## MATERIALS AND METHODS

The presence of pigments in plant tissues gives color to vegetables and fruit, which is different depending on variety and species. Pigments are substances with very different chemical structure; they are present in the form of porphyrin pigments, carotenoids, anthocyanin and flavonoids. The main porphyrin pigments found in vegetables are chlorophyll a, b and c. Chlorophyll a, the main pigment in plants, converts light energy into chemical energy through photosynthesis process. The content of chlorophyll pigments varies by species.

### Chemicals

All the chemicals used throughout the study were of analytical grade. All the glass wares used were sterilized prior to use.

### Experimental

#### Plant Material

The wheat, spinach, and eupatorium plants were collected from the local market of Dehradun. The plant material was rinsed with cold water. The plants were then cut into small pieces separately and the extraction was carried out by decoction technique. Plant samples were prepared with a laboratory homogenizer using about 50 g fresh material. Three extraction solutions were used for each sample: 95% aqueous diethyl ether solution (petroleum ether), 90% aqueous methanol solution and 100% acetone. The extraction ratio was 1:50. Homogenized mixture is separated by centrifugation at 3000 rpm, for 10 minutes. The analytical determination was performed with visible spectrophotometer at the following wavelengths: 645, 653, 662 and 664 nm, for chlorophyll a and b (according to each extraction solvent) and 470 nm for carotene.

The homogenized mixture was then filtered through whatmann filter paper. The filtrate so obtained was then subjected to the determination of various phytochemicals like chlorophyll, carotenoids, glucose, ascorbic acid, proteins.

Equations used for calculation are presented below.

#### Diethyl ether

Chlorophyll a =  $10.05 A_{662} - 0.766 A_{644}$

Chlorophyll b =  $16.37 A_{664} - 3.140 A_{662}$

Carotene =  $1000 A_{470} - 1.280 \text{ Chl a} - 56.7 \text{ Chl b}/230$ .

#### Methanol

Chlorophyll a =  $15.65 A_{666} - 7.340 A_{653}$

Chlorophyll b =  $27.05 A_{653} - 11.21 A_{666}$

Carotene =  $1000 A_{470} - 2.860 \text{ Chl a} - 129.2 \text{ Chl b}/245$ .

#### Acetone

Chlorophyll a =  $11.75 A_{662} - 2.350 A_{645}$

Chlorophyll b =  $18.61 A_{645} - 3.960 A_{662}$

Carotene =  $1000 A_{470} - 2.270 \text{ Chl a} - 81.4 \text{ Chl b}/227$ .

### Procedure for the determination of Ascorbic Acid

Five grams of sample were homogenized with 25 ml of metaphosphoric acid acetic acid solution, and it was quantitatively transferred into a 50 ml volumetric flask and shaken gently to homogenized 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. Spectrophotometric determination (perkin elmer spectrophotometer lambda) of vitamin C content in nine different samples of plant extract.

### Estimation of Vitamin C

#### Procedure

0.23 ml of 3% bromine water were added into 4 ml of centrifuged sample solution to oxidize the ascorbic acid to dehydro ascorbic acid and after that 0.13ml of 10% thio urea 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 5ml chilled 85% H<sub>2</sub>SO<sub>4</sub> with constant stirring. As a result, a coloured solution's absorbance was taken at 521 nm.

### Procedure for the Determination of Glucose

#### Materials Required

Phenol 5%, redistilled phenol (50gm) dissolved in water and diluted to 1 liter sulphuric acid 96% reagent grade.

#### Standard Glucose

Stock- 100 mg in 10 ml H<sub>2</sub>O.

#### Working Standard

10ml of stalk diluted to 100ml with distilled water.

#### Principle

In hot acidic medium, glucose is dehydrated to hydroxyl methyl. This forms a green coloured product with phenol and had a absorption maximum at 490nm.

#### Procedure

1. Weigh out 100mg of the sample in a boiling tube.
2. Hydrolyze by keeping it in a boiling water bath for three hours with 5ml of 2.5 NaCl and cool it to room temperature.

3. Neutralize it with solid sodium carbonate until the effervescence ceases.
4. Make up the volume to 100 ml and centrifuge.
5. Pipette out 0.2, 0.4, 0.6, 0.8, and 1ml of the working standards into a series of test tube.
6. Pipette out 0.1 and 0.2ml of the sample solution in two separate test tubes make up the volume in each test tube to 1ml with H<sub>2</sub>O.
7. Set a blank with 1ml of H<sub>2</sub>O.
8. Add 5ml of 96% sulphuric acid to each test tube and shake well.
9. Add 1ml of phenol solution to each test tube.
10. After 10 min shake the contents in the test tube and place in a water bath at 25<sup>0</sup> -30<sup>0</sup> C for 30 minutes.
11. Read the colour at 490 nm.
12. Calculate the amount of total carbohydrates present in the standard solution using the standard graph.

#### **Procedure for the Determination of Amino-Acids by Ninhydrin Method**

The amino acids are colourless ionic compounds the forms the basic building blocks of proteins. Apart from being bound as proteins, amino acids also exist in the free form in many tissues and are known as free amino acid. They are mostly water soluble in nature. Very often in plants during disease conditions the free amino acid composition exhibits a change hence, the measurement of the total free amino acids gives the physiological and health states of the plants.

#### **Principle**

Ninhydrin, a powerful oxidizing agent, decarboxylates the alpha amino acids and yields an intensity coloured bluish purple product which is spectrophotometrically measured at 570nm.

#### **Reagents Required**

1. Ninhydrin:- dissolve 0.8gm stannous chloride (SnCl<sub>2</sub>.2H<sub>2</sub>O) in 500ml of 0.2M citrate buffer(pH 5.0) add this solution to 20gm of ninhydrin in 500ml of methyl cellosolve (2methoxy ethanol).
2. 0.2M Citrate Buffer pH5.0 Solution A: 0.2M citric acid. Solution B: 0.2M sodium citrate

mix 20.5ml of solution A with 29.5ml of solution B and check pH.

3. Dilute Solvent: mix equal volume of water and n-propanol and use.
4. Standard: Dissolve 50mg leucine in 50ml of distilled water in a volumetric flask. Take 10ml of this stock standard and dilute to 100ml in another flask for working standard solution.

#### **Procedure**

1. Pipette out 0.2, 0.4, 0.6, 0.8, 1.0 ml of standard amino acid solution to the resp. labeled test tubes.
2. Add distilled water in all the test tubes and make up the volume to 1ml.
3. Add 1ml of distilled water to the test tube to make up the volume to 1ml.
4. Now add 1ml of ninhydrin reagent to all the test tube including the blank test tube and unknown test tube.
5. Mix the contents of the test tubes by shaking the tubes.
6. Then cover all the test tubes with papers.
7. Place all the test tubes in boiling water bath for 15minutes.
8. Cool the test tubes in cold water and add 5ml of diluents solvents to each test tube and mix well.
9. Now record the absorbance at 570nm of each solution using a colorimeter.
10. Then plot the standard curve by taking concentration along X axis and absorbance at 570nm along Y-axis.

#### **RESULTS AND DISCUSSION**

The present study carried out on the *Triticum aestivum*, *Spinacia oclera*, and *Eupatorium odoratum* revealed the presence of medicinal active constituents. The chlorophyll contents of *Triticum aestivum*, *Spinacia oclera*, *Eupatorium odoratum* were qualitatively analyzed and the results are presented in Table No.1, 2 and 3. As per quantitative analysis carried out for glucose, protein and ascorbic acid estimation the results obtained are quite interesting as they shows different types of results in

different solvents. The plants under study are edible and bear a good nutritive potential. In addition the medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body like ascorbic acid as being involved in the strengthen of teeth and in help in the protection against bone cancer. The chlorophyll (a) helps in the vision process and help to maintain other physiological function in the body like regular regulation of stomach thereby help the intestines to absorb the daily in taken metabolites and the elimination of the waste. They also help to maintain the electrostatic potential across the cell membrane. Same goes for chlorophyll (b) and carotenoids. Carotenoids have a major role in the synthesis of carotene for vision process, and acting as a good supplement for the development of body cells.

Similarly the glucose and the protein content in the concerned plants reveal a good utilization of the plants for its consumption as a vegetable.

The chlorophyll (a) content of *Triticum aestivum*, *Spinacia oclera*, *Eupatorium odoratum* in acetone extract was found to be (8.75, 0.480, 0.31 g/l). In methanol extracts the contents were found to be (6.89, 3.49, 3.80) and in the diethyl ether extract the chlorophyll (a) content was found to be (0.875, 0.45, 1.075) respectively. As per the results obtained the chlorophyll (a) was found highest in acetone and methanol extract of *Triticum aestivum*, *Spinacia oclera* followed by *eupatorium odoratum*. But the diethyl ether extract of *eupatorium posses* more chlorophyll (a) content than that of *Triticum aestivum*, *Spinacia oclera* (Figure No.1).

The chlorophyll (b) content of *Triticum aestivum*, *Spinacia oclera*, *Eupatorium odoratum* extract in acetone extract was found to be (7.45, 7.05, 7.18 g/l) Methanol extracts the contents were found to be (2.56, 2.45, 2.15 g/l) and in the diethyl ether extract the chlorophyll (b) content was found to be (3.95, 2.55, 6.66 g/l) respectively. As per the results obtained the chlorophyll (b) content was found highest in the acetone extract of *Triticum aestivum* followed by diethyl ether extract of *Eupatorium odoratum* (Figure No.2).

The carotenoid content of *Triticum aestivum*, *Spinacia oclera*, *Eupatorium odoratum* extract in acetone extract was found to be (1.04, 1.93, 1.96 g/l) Methanol extracts the contents were found to be (0.56, 0.46, 1.76 g/l) and in the diethyl ether extract the carotenoid content was found to be (0.16, 0.31, 0.89) respectively. The carotenoid content was found highest in all the acetone extracts of the concerned three plants followed by methanol extract, and fewer contents were found present in diethyl ether extracts of all plants (Figure No.3).

The glucose content of *Triticum aestivum*, *Spinacia oclera*, *Eupatorium odoratum* extract in acetone extract was found to be (159.39, 56.96, 120.30 x 10<sup>-3</sup> g/l). Methanol extracts the contents were found to be (174.54, 123.33, 175.75 x 10<sup>-3</sup> g/l) and in the diethyl ether extract the glucose content was found to be (19.39, 2.42, 97.57 x 10<sup>-3</sup> g/l) respectively. The glucose content was found highest in the acetone extracts followed by methanol extracts and lesser amount of carotenoids was found present in the diethyl ether extracts (Figure No.4).

The Ascorbic acid content of *Triticum aestivum*, *Spinacia oclera*, *Eupatorium odoratum* extract in acetone extract was found to be (0.140, 0.150, 0.230 x 10<sup>-3</sup>g g/l) Methanol extracts the contents were found to be (0.090, 0.089, 0.088 x 10<sup>-3</sup>g/l) and in the diethyl ether extract the ascorbic acid content was found to be (0.090, 0.254, 0.107 x 10<sup>-3</sup>g) respectively. Among the *Triticum aestivum*, *Spinacia oclera*, *Eupatorium odoratum*, the ascorbic acid was found highest in acetone extracts followed by methanol extracts and finally fewer in the diethyl ether extracts (Figure No.5).

The protein content of *Triticum aestivum*, *Spinacia oclera*, *Eupatorium odoratum* in acetone extract was found to be (12.5, 65.00, 53.5 x 10<sup>-3</sup>g/l) Methanol extracts the contents were found to be (24.0, 44.00, 13.5 x 10<sup>-3</sup>g/l) and in the diethyl ether extract the protein content was found to be (37.5, 105.0, 10.0 x 10<sup>-3</sup>g/l) respectively. The protein content was found highest in the diethyl ether extract *Spinacia oclera* followed by acetone extract and little amount was found in methanol extract of the concerned plants (Figure No.6).

**Observation**

The Data Sheet for Determination of Chlorophyll Contents are shown below

**Table No.1: Graph showing Chlorophyll (A) Content in various solvents**

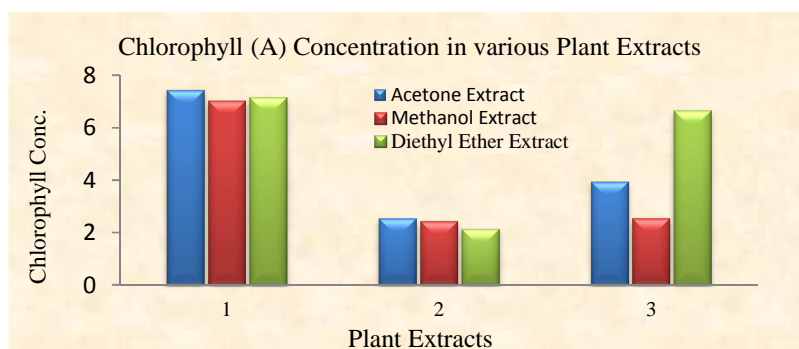
S.No	Name of the plant	Chlorophyll a (g/l)		
		Acetone	Methanol	Diethyl ether
1	<i>Triticum aestivum</i>	8.75	6.89	0.875
2	<i>Spinacia oclera</i>	0.180	3.49	0.45
3	<i>Eupatorium odoratum</i>	0.31	3.80	1.075

**Table No.2: Graph showing Chlorophyll (b) Content in various solvents**

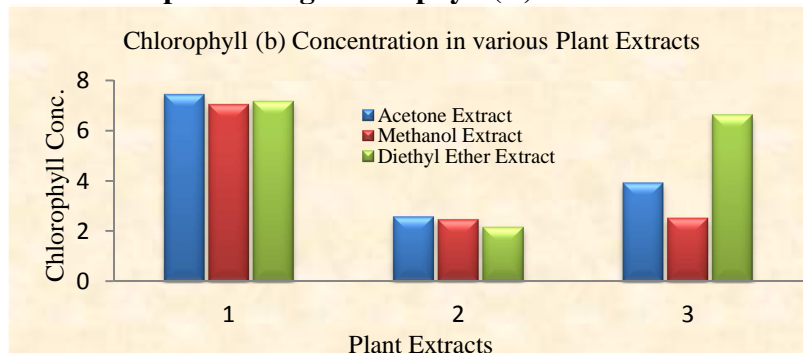
S.No	Name of the plant	Chlorophyll b (g/l)		
		Acetone	Methanol	Diethyl ether
1	<i>Triticum aestivum</i>	7.45	2.56	3.95
2	<i>Spinacia oclera</i>	7.05	2.45	2.55
3	<i>Eupatorium odoratum</i>	7.18	2.15	6.66

**Table No.3: And Graph showing Carotenoid content in various solvents**

S.No	Name of the plant	Carotenoids (g/l)		
		Acetone	Methanol	Diethyl ether
1	<i>Triticum aestivum</i>	1.04	0.56	0.16
2	<i>Spinacia oclera</i>	1.93	0.46	0.031
3	<i>Eupatorium odoratum</i>	1.96	1.76	0.89



**Figure No.1: Graph showing Chlorophyll (A) Content in various solvents**



**Figure No.2: Graph showing Chlorophyll (b) Content in various solvents**

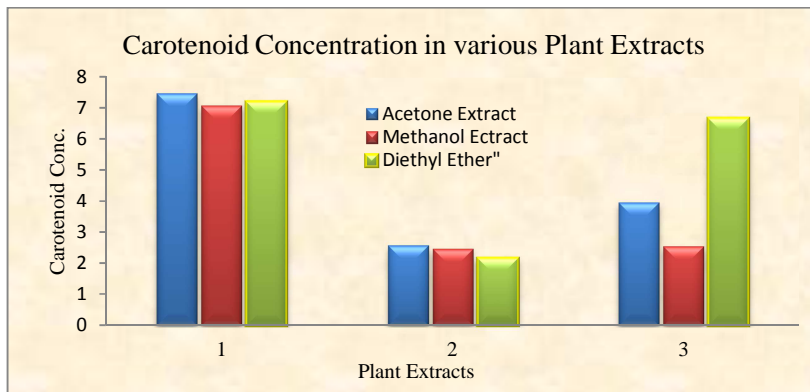


Figure No.3: Graph showing Carotenoid content in various solvents

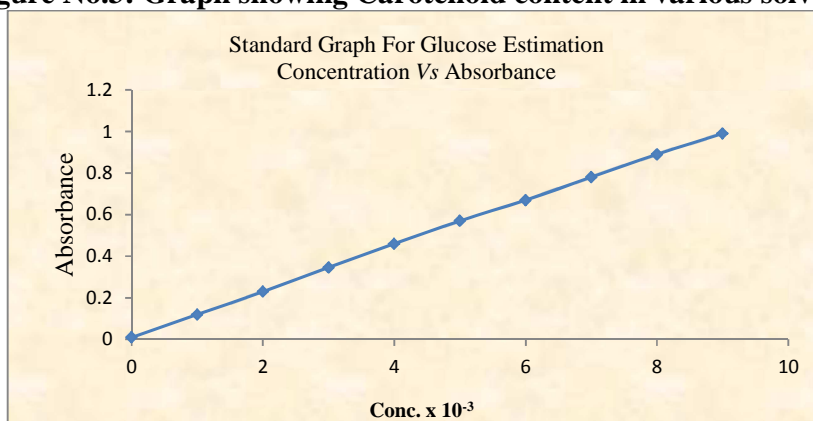


Figure No.4: Standard Graph for the Determination of Glucose

From the Standard Graph the value of (a) as derived from Beer-Lambert Law comes out to

$$A = a \cdot c \cdot t$$

$$a = 2.0 \times 10^{-3} \text{ g/L}$$

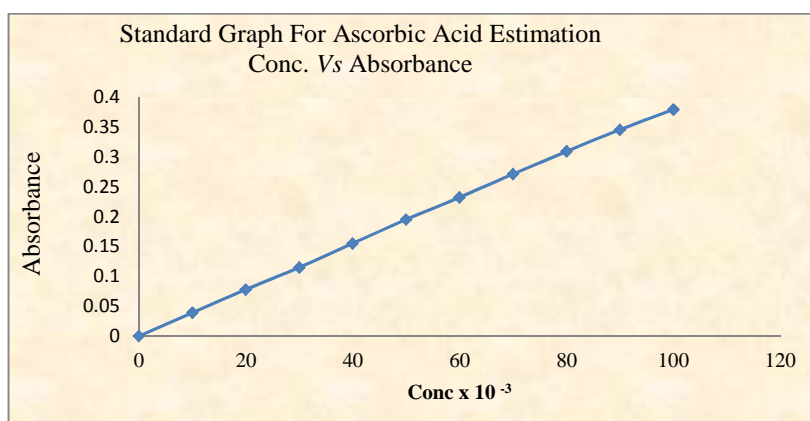
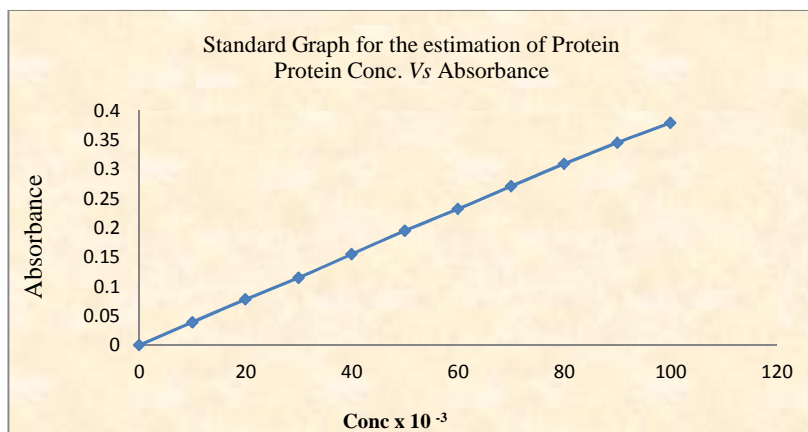


Figure No.5: Standard Graph for the Determination of Ascorbic Acid

From the Standard Graph the value of (a) as derived from Beer-Lambert Law comes out to

$$A = a \cdot c \cdot t$$
$$a = 3.3 \times 10^{-3} \text{ g/L}$$



**Figure No.6: Standard Graph for the Determination of Protein**

From the Standard Graph the value of (a) as derived from Beer-Lambert Law comes out to

$$A = a \cdot c \cdot t$$
$$a = 0.9 \times 10^{-3} \text{ g/L}$$

## CONCLUSION

As per the result obtained from these concerned plant species, it was found that all the three plant species bear a good nutritive value. In case where different solvents furnish different nutritive value is due to the interaction of these components with the concerned solvent differently, so different components have different type of concentrations in the respective solvents, which could be due to polarity index, hydrogen bonding and other mechanisms. From the above results it could be concluded that all the three concerned plants should be consumed regularly and as per the bodily demand as they boost up the bodily mechanisms by one way or another way. The secondary metabolites like chlorophyll help in the maintenance of the primary metabolites. The ascorbic acid content, glucose and protein content in the three plant species is within the range of consumption, so are useful.

## ACKNOWLEDGEMENT

All authors are would like to thanks Uttaranchal College of Science and Technology, Dehradun, India for continuous support and encouragement throughout this research work.

## CONFLICT OF INTEREST

None declared.

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**Please cite this article in press as:** M. Amin Mir et al. Analysis on the Food Value and Chlorophyll Contents of Various Plant Species of Dehradun, *Asian Journal of Research in Biological and Pharmaceutical Sciences*, 2(4), 2014, 150-158.