The Plants were identified and authenticated from Botanical Survey of India (BSI), Northern Regional Centre, Dehradun, India.

2.2. Estimation of Moisture Content and Dry Matter (DM)

Sample was grinded, and 25 gm of the grinded sample was taken in weighed petridish. Then sample was kept in a hot-air oven for 24 hours at 100 ± 5°C. The dried sample was cooled in a desiccator and weighed to a constant weight. The loss in weight was considered as the moisture percentage and was calculated by using following formula:

$$\% \text{ of D.M.} = \frac{W2 - W3}{W2 - W1} \times 100$$

Where; W_1 = wt. of an empty petridish, W_2 = wt. of petridish with sample before hot-air oven dry and W_3 = wt. of petridish with dried sample. The remaining amount was considered as moisture content.

2.3 Preliminary Screening of Phytochemicals

Preliminary Screening of Phytochemicals was carried out as per the procedure laid down by WHO [14]. Samples were evaluated for the presence of various phyto-constituents covering primary and secondary metabolites like flavonoids, phenols, alkaloids etc.

2.4 Determination of Carbohydrates

Total carbohydrate content in *A. tuberosum* was determined by Anthrone method [15]. 100 mg of the powdered sample was hydrolyzed in 5.0 ml of 2.5 N HCl by keeping it in a boiling water bath for three hours. It was then neutralized with sodium carbonate until the effervescence was ceased. Volume was then finally made to 100 ml and centrifuged. 0.5 and 1.0 ml aliquots were taken from the supernatant for analysis. Meanwhile, standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard. Volume was made up to 1.0 ml including the samples. Then 4.0 ml of ice cooled anthrone reagent was added and heated for eight minutes in a boiling water bath. It was then cooled rapidly and finally the green to dark green colour was read at 630 nm. Based on the standard curve the amount of carbohydrate present in the sample tube was calculated.

2.4.1. Determination of Polysaccharides

Polysaccharide content in *A. tuberosum* samples was measured by extracting all simple sugars with the help of hot 80% ethyl alcohol. The remaining polysaccharide content was measured spectrophotometerically using anthrone reagent.

2.4.2. Determination of Sugars

Simple soluble sugars were measured by subtracting the amount of polysaccharides from the total amount of carbohydrates present in the sample [15].

2.4.2.1 Détermination of Reducing Sugars

Estimation of reducing sugars was carried out using Dinitrosalicylic acid (DNS) method [16]. 100 mg of the sample was weighed and the sugars were extracted with hot 80% ethanol twice (5.0 ml each time). Supernatant collected was then evaporated on a boiling water bath at 80°C and then 10 ml water was added to dissolve the sugars. 0.5 to 3.0 ml of the extract was pipette out in test tubes and the volume was made to 3.0 ml with water in all the tubes, 3.0 ml of DNS reagent was added, heated the contents of the tubes till they were warm, 1.0 ml of 40% Rochelle salt solution was added. When the contents of the tubes were still warm, 1.0 ml of 40% Rochelle salt solution was added, mixtures were cooled and finally the intensity of dark red colour at 510 nm was read. And also a series of standards was run using glucose (0-500 µg) and finally plotted a graph. The amount of reducing sugars present in the sample was calculated by using the standard graph using D-glucose as a standard.

2.4.2.2 Determination of Non- reducing sugars

Non-reducing sugar content in *A. tuberosum* was calculated by simply subtracting the amount of reducing sugars from the total amount of the simple sugars present in the sample taken.

2.5 Determination of crude proteins

Dried sample (150 mg) was taken into Kjeldahl's digestion tube in which 10 gm. of digestion mixture (9.5 gm. of Potassium sulphate and 0.5 gm. of Copper sulphate) and 20 ml of concentrated Sulphuric acid were added. The mixture was digested at 440°C for 40 minutes. Tubes were allowed to be cooled at room temperature. The standard Kjeldahl procedure was adopted using the automated Kjeltec nitrogen and protein analyzer unit (Kjeltec-2300, Foss Tecator, Switzerland).

2.6 Determination of Ether-Extract

Estimation of ether-extract was done with the help of Soxhlet's apparatus [17]. Oven dried sample (5 gm.) was taken in a thimble of Whatman filter paper No. 1 and placed in the extractor. The extractor was connected with weighed oil flask below and the condenser above. Petroleum ether of B.P. 60 to 80°C was poured into the extraction tube with 60 ml. more than required for permitting siphon to the oil flask placed on the heater. Cold water was passed through the condenser during the extraction process. Extraction was carried out for 6 hours till the liquid was as clear as clean water. The flask was then disconnected and dried in a hot air oven at 100 ± 5°C for 4 to 6 hours till the ether was completely evaporated. It was cooled in a desiccator and weighed to a constant weight. The difference in the weight of oil flask after and before extraction denoted the ether extract of the sample.

2.7 Estimation of Chlorophyll content

Allium tuberosum sample powder was assessed for chlorophyll a and chlorophyll b. The weighed samples was put in acetone (50 ml for each gram) and homogenized. The homogenate was filtered through two layer cheese cloths, and was centrifuged at 2500 rpm for ten minutes. The supernatant was separated and the absorbance was read at 400-700 nm on Labomed double beam spectrophotometer. It was recorded that Chlorophyll a showed the maximum absorbance at 662 nm and chlorophyll b at 646 nm and the

amount of these pigments was calculated according to the formulas of Lichtentaler and Wellburn [18].

$C_a = 11.75\ A_{662} - 2.350\ A_{645}$
$C_b = 18.61\ A_{645} - 3.960\ A_{662}$

2.8 Determination of Crude fibres

The sample after ether extraction was transferred from the thimble to a 500 ml. round bottom reflux condenser flask. 200 ml. of 1.25% Sulphuric acid solution was poured into the flask and was heated in a heating mantle. Cold water was allowed to flow through the condenser. After boiling for 30 minutes, the content of the flask was filtered through the muslin cloth. The residue on the cloth was washed with distilled water to remove the acid.

The residue was transferred to the same flask and 200 ml. of 1.25% Sodium hydroxide solution was poured into the flask. The content was boiled for 30 minutes and filtered through the same cloth. It was washed with distilled water to remove the alkali. The residue was transferred to a crucible and kept in a hot-air oven at 100 ± 5°C for drying. The crucible was cooled in a desiccator and weighed to a constant weight. The content was ashed in a muffle furnace at 600°C (ash should be free of black particles), cooled in a desiccator and weighed [19].

2.9 Ash value

It was calculated by using a standard method [14]. Dried sample (5.0 g) was taken in a weighed crucible and heated in a muffle furnace at 600 ± 15°C till the content was free of black particles. The crucible was cooled in a desiccator and weighed to a constant weight. The ash content was calculated by weighing the weight of crucible at different stage. The ash remaining following ignition of medicinal plant materials is determined by three different methods which measure total ash, acid-insoluble ash and water-soluble ash.

2.9.1 Acid insoluble ash
Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth.

Procedure: To the crucible containing the total ash, 25 ml of the hydrochloric acid was added covered with a watch glass and boiled gently for 5 minutes. The watch glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble mass was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on the hot plate and ignited to the constant weighed. Allowed the residue to cool in a suitable dissicator for 30 minutes, then weighed without delay. The content of acid insoluble ash was calculated with reference to the air dried sample of the crude drug [14].

2.9.2 Water Soluble Ash
Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.
Procedure: To the crucible containing the total ash 25 ml of water was added and boiled for 5 minutes. The insoluble matter was collected in the sintered glass crucible or on an ashless filter paper, washed with hot water and ignited the crucible for 15 minutes at a constant temperature not exceeding 450ºC. The weight of the residue was subtracted from the weight of the total ash, the difference in weight represent the water soluble ash. The content of water insoluble ash was calculated with reference to the air dried material [14].

2.10 Statistical Analysis

Results are expressed as mean ± Standard Deviation (SD) of triplicate experiments. The data were subjected to one-way analysis of variance (ANOVA), and significant differences between means were determined statistically using Student's 't' test with the OpenStat software. $P < 0.05$ was assumed as the level of significance for experimental results.

3. Results and Discussion

3.1. Moisture Content and Dry Matter (DM)

The fresh samples of *A. tuberosum* were analyzed for Moisture Content and Dry Matter. Samples were found to contain 92.18 ± 0.36 % moisture content, and the dry weight was reported 7.82 ± 0.36 g/100gfw.

3.2. Preliminary Screening of Phyto-chemicals

A. tuberosum samples were qualitatively investigated for their primary and secondary metabolites. Samples showed the presence of following biologically active phyto-constituents (Table-1).

Table 1: Phyto-chemical screening of A. tuberosum.

S. No.	*Plant Constituents*	*Water*	*Methanol*	*Ethyl acetate*	*Hexane*
1.	Alkaloids	+	+	-	-
2.	Phenolic compounds	+	+	-	-
3.	Glycosides	+	+	-	-
4.	Proteins	+	+	-	-
5.	Saponins	+	+	-	-
6.	Flavonoids	+	+	-	-
7.	Tannins	+	+	-	-
8.	Steroids	-	-	+	+
9.	Carbohydrates	+	+	-	-

Where (+) indicate presence and (-) indicates absence of phyto-constituents

3.3 Carbohydrates

Carbohydrates are the macromolecules primarily required for our daily energy need. The chemical formula of carbohydrates is $C_n(H_2O)_y$,where $y \leq n$. Carbohydrates can be classified as monomer like glucose, fructose or dimer like sucrose or polymers like polysaccharide molecules,starch, cellulose etc. In the present investigation we were focused

on to quantify the total carbohydrate content, sugars, polysaccharides, reducing and non-reducing sugars. Based on the calibration curve y = 0.007x, R^2 =0.999, the total carbohydrate content in *A. tuberosum* was found 2.75 ± 0.16 %, out of which 1.65 ± 0.13 % was polysaccharide content. Polysaccharides are macromolecules having the beta lactogens unit which are reported to be anti-cancerous in the literature. Based on calibration curve straight line equation y = 0.010x, R^2 =0.996, it is observed that *A. tuberosum* samples are having 0.48 ± 0.08 % reducing sugar and 0.62 ± 0.08 % non reducing sugar

3.4 Proteins

Proteins are the next abundant bio-molecule after carbohydrates which are required for body building and body maintenance. Protein molecules are made up of various amino acids. The essential amino acids of a plant source have always been an important factor for protein intake from outer sources. In present investigation the crude protein content in *A. tuberosum* was quantified and it was found 2.25 ± 0.14 g/100gfw.

3.5 Ether extracts

Ether extracts are composed of non polar bio-molecules like fats and other lipid molecules. *A. tuberosum* was found to contain 0.1722 ± 0.03 g/100gfw of ether extract. Fats can be classified as saturated and unsaturated fats. Unsaturated fats are divided into monounsaturated fats and polyunsaturated fats, and both types are thought to have beneficial effects on cholesterol levels. Monounsaturated fats help lower LDL (bad) cholesterol while also boosting HDL (good) cholesterol.

3.6 Estimation of Chlorophyll content

Chlorophyll is the complex bio-molecule responsible for photosynthesis in the plant. *A. tuberosum* leaves were extracted for chlorophyll contents viz. chlorophyll 'a' and chlorophyll 'b'. The results showed that it contains 0.32 ± 0.04 mg/100gfw chlorophyll 'a' and 0.26 ± 0.03 mg/100gfw chlorophyll 'b'

3.7 Fibres

Fibre is a structural unit of thick-walled plant cell. They are very important in the biology of both plants and animals, for holding tissues together. Fibres are very important for digestive tract as they increase the surface area of food and make the digestion process easier. In the present investigation the *A. tuberosum* was found to contain 0.7783 ± 0.09g/100gfw.

3.8 Ash value

Ash content of a plant material represents the total amount of non volatile mineral oxides and hydroxides along with thermally stable mineral salts and carbon content. The ash content contains many nutritionally important macro and micronutrients, which are very helpful for curing many diseases based on mineral deficiencies. In the present study, we were focused on to quantify the total ash content in *A. tuberosum* along with the proportions like Water soluble ash, Water insoluble ash, Acid soluble ash and Acid insoluble ash as per the WHO guidelines [14]. The total ash content was found 0.8142g/100gfw, out of which 0.7477g/100g was found to be acid soluble and 0.4287g/100g water soluble in different experiments. (Fig. 1).

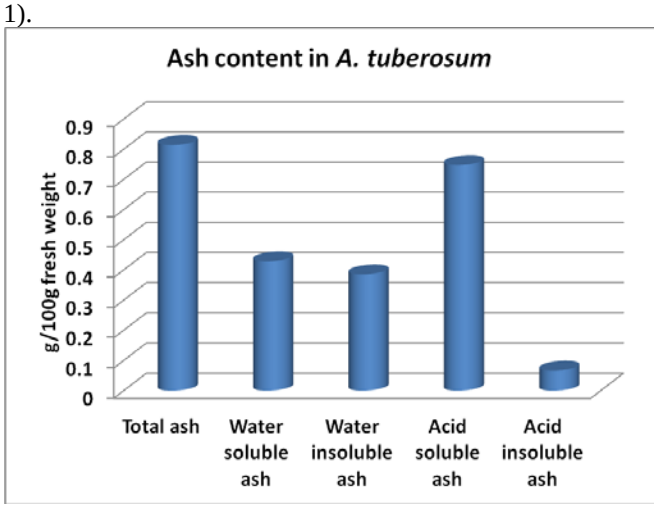

Figure 1: Ash content in *A. tuberosum*

The overall view of nutritional value of *A. tuberosum* on the fresh weight basis of the samples can be seen, as given below (Table-2 and Fig. 2).

Table 2: Nutritional analysis of *A. tuberosum*

S.No.	*Biochemical Parameters*	*Quantity in 100gfw*
1.	Moisture content	92.18 ± 0.36 %
2.	Carbohydrates	2.75 ± 0.16 g
3.	Polysaccharides	1.65 ± 0.13 g
4.	Total soluble sugars	1.10 ± 0.10 g
5.	Reducing sugars	0.48 ± 0.08 g
6.	Non Reducing sugars	0.62 ± 0.08 g
7.	Crude proteins	2.25 ± 0.14 g
8.	Ether extract	0.1722 ± 0.03 g
9.	Chlorophyll a	0.32 ± 0.04 mg
10.	Chlorophyll b	0.26 ± 0.03 mg
11.	Crude fibers	0.7783 ± 0.09g

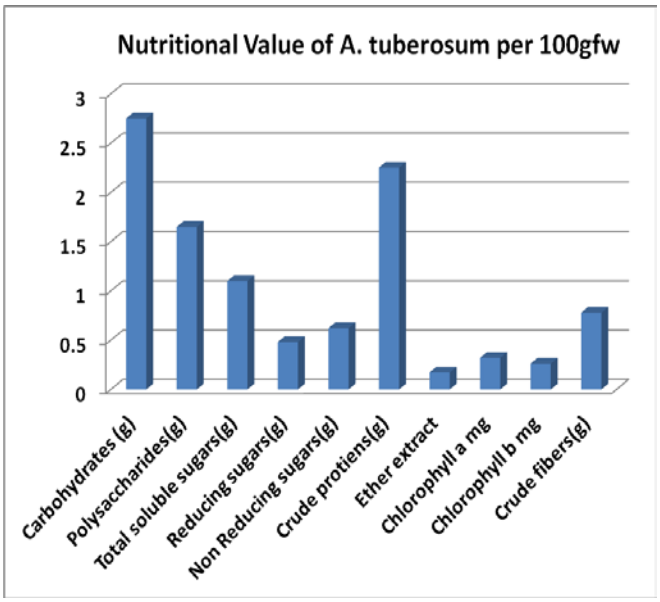

Figure 2: Nutritional value of *A. tuberosum* (Fresh weight basis)

4. Conclusion

Nutritional screening reveals the importance of *A. tuberosum* in the growing realm of nutraceutical and pharmaceutical industries. The perceptive amounts of bio-molecules present in *A. tuberosum* are found to be very useful for healthy life. Although the sulphur compounds are primary bioactive ingredient in this plant, but its pharmacological activity is also attributed to the presence of polysaccharides. In this study it is found that the level of polysaccharides present in fresh samples of *A. tuberosum* was 1.65 ± 0.13 g % and therefore it is worthwhile to conclude that *A. tuberosum* can be a very useful herb in treating various ailments, which can be cured through polysaccharide content. Its protein content (2.25 ± 0.14 %) and fibrous content (0.7783 ± 0.09 %) makes this herb, a healthy food. Its carbohydrate content (2.75 ± 0.16 %) and ash value (0.8142g/100 gfw) are very good indicators of its nutritional value.

In view of the above, it is worthwhile to say that *A. tuberosum* is under exploited high valued medicinal herbal plant, which can be taken as a daily health supplement either as nutrient or as traditional herb. There is a lot of scope for the future research on the various aspects of its cultivation, biochemistry, pharmacology and pharmacognosy. Bio-metabolites like polysaccharides, fibres, phenolic compounds and alike materials of *A. tuberosum* will be the key future driving force in the domain of nutraceutical industries.

5. Acknowledgement

Authors pay their sincere gratitude to Dr. M. Nasim, Director, Defence Institute of Bio-Energy Research, Haldwani for providing laboratory facilities. The encouragement from Principal and Dr. A.N. Sah, Kumaun University, Nainital is also well acknowledged. Special thanks goes to Dr. P. S. Negi, Scientist 'E', Dr. H. K. Pandey, Scientist 'D', Ms. Seema Singh, Senior Research Fellow and Mr. Subir Ranjan, Technical Officer 'A' Defence Institute of Bio-Energy Research, Field Station, Pithoragarh, Uttarakhand, India for their outstanding support to this study. Authors also acknowledged the help of Botanical Survey of India (BSI) Northern Regional Centre, Dehradun, India for Plant identification and authentication.

References

[1] Moyers S. Garlic in Health, History & World Cuisine, *Suncoast Press*. 1996; 1-36.
[2] Lawson LD, Bauer R. Garlic: A review of its medicinal effects and indicated active compounds. *American Chemical Society*. 1998; 176–209.
[3] Orekhv AN and Grunwald J. Effects of garlic on atherosclerosis, *Nutrition Journal*. 1997;13: 656.
[4] Roman O, Pino ME, Pereda T, Valuenzuela A. Effects of pindolol and propranolol on blood lipids in hypertensive patients Cardiovasc. *Drugs therapy*. 1989; 3: 767.
[5] Simons LA. Additive effect of plant sterolester margarine - cerivastatin in lowering low density lipoprotein cholesterol in primary hypercholesterolemia. *Am. J. Cardiol*. 2002; 90: 757.
[6] Riggs DR, Lamm DL. Garlic: Treatment for cell carcinoma. 1997, 1666-1690.
[7] Banerjee SK, Maulik S. Effect of garlic on cardiovascular disorders. *Nutrition Journal*. 2002; 1: 4-30.
[8] Cavallito CJ, Bailey JH. Allicin – The antibacterial principle of Allium sativum - isolation, physical properties and antibacterial action. *Am. J. Chem. Soc*. 1944; 1950–1954.
[9] Kendler BS. Garlic & Onion: Review of relation to cardiovascular disease. 1997; 670-685.
[10] Saghir AR, Mann LK, Bernhard RA, Jambsen JV. Determination of aliphatic mono and disulphides in *Allium* by gas chromatography and their distribution in the common food species. *Proc. Amer. Soc. Hort. Sci*. 1964; 84: 386-98,
[11] Cavallito CJ, Bailey JH. Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. *Journal of the American Chemical Society*. 1944; 66: 1950-1951.
[12] Shah NC. Status of cultivated & wild *Allium* species in India : *The Scitech journal*. 2014; 09 (01): 28-36.
[13] Alizadeh B, Royandazagh SD, Khawar KM Ozcan S. Micropropagation of garlic chives (*Allium tuberosum* rottl. ex Sprang) using mesocotyl axis. *The Journal of Animal & Plant Sciences*. 2013; 23(2): 543-549.
[14] WHO, Quality Control Methods for Medicinal Plant Material. World Health Organization, Geneva, ISBN:9789241545105. 1998; 115.
[15] Sadasivam S, Manickam A. Biochemical methods, 2nd edn. New Age International Publication, 1996; 8-21.
[16] Miller, GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar, *Anal. Chem*. 1959; 31: 426.
[17] Franz Ritter von Soxhlet, Die gewichtsanalytische Bestimmung des Milchfettes, *Dingler's Polytechnisches Journal*. 1879; 232: 461-465.
[18] Lichtenthaler HK, Wellburn AR. Determination of total carotenoids and chlorophyll a and b of leaf extract in different solvents. *Biochem. Soc. Trans.*, 1983; *11*: 591-592.
[19] Maynard AJ. (Ed.) Methods in Food Analysis, Academic Press, New York. 1970; 176.