

Comparative Study of Phytochemicals Compounds of Medicinal and Aromatic Herbs under Different Growing Medium

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ABSTRACT

Phytochemicals are those compounds which found naturally in plants. The compounds play an important role in plant metabolic activity and in human health by protecting from many diseases. These compounds provide plant protection from many diseases. Globally, hydroponics technology has been accepted as sustainable technology to tackle all issues being reported in conventional system. Hydroponics not only offers safe food but also ensures vertical utilization of space with higher water use efficiency. Soil is the natural growing media which provide potential support to plant growth and development. Soilless is the growing media without using soil. Himalayan seasoning *Allium* or Stracheys Chive *Allium stracheyi* is a member of Amaryllidaceae family. Vernacular name is "Fern, Jambu". *Allium stracheyi* is enlisted as Vulnerable in the Red Data Book of Indian Plants and also categorized it as Vulnerable in the western Himalayan states of Jammu & Kashmir, Himachal Pradesh, and Uttarakhand. One popular culinary herb is sweet basil (*Ocimum basilicum* L.). In Hindi it is known as Babaui Tulsi. Among various *Ocimum* species, *Ocimum basilicum* is commercially and extensively cultivated for essential oil production. Its oil is employed for flavouring of food stuffs, confectionery, condiments, and perfumery industry. Pepper Mint (*Mentha piperita*) is the aromatic, herbaceous plant used for menthol extraction it belongs to the family Lamiaceae. This experiment was conducted during 2021-2022 at DIBER, DRDO, Haldwani, Nainital (Uttarakhand). The objective of this study was to evaluate the Phytochemical properties of different medicinal and

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aromatic herbs under different growing conditions. Among different growing condition jambu crop exhibited the tannin and flavonoid content higher (235.42 mg TAE/100 g, 10.44 mg QE/g respectively) under hydroponic growing condition as comparison to other growing condition.

Whereas the total phenol compound found higher (623.44 mg GAE/100 g) under soil growing condition. In Mint aromatic plant the total phenol and flavonoid attributes found higher (632.75 mg GAE/100 g, 25.34 mg QE/g respectively) under soilless growing condition whereas the tannin compound determined higher (198.49 mg TAE/100 g) under hydroponics growing condition. The quality attributes in sweet basil found that's the tannin and flavonoid recorded higher (301.10 mg TAE/100 g, 19.00 mg QE/g respectively) under soilless growing media as compared to other growing conditions. Whereas the total phenol content found higher (569.13 mg GAE/100 g) under soil growing media. The ascorbic acid found in jambu herb (34.81 mg/100 g) ascorbic acid under soil growing condition, whereas it is found in mint and sweet basil (11.0 mg/100 g, 11.09 mg/100 g respectively) higher under hydroponics growing condition as compared to other growing condition.

Keywords: Hydroponics; Flavonoid; Medicinal herbs; Phytochemicals; Diseases; Nutrients; Antioxidant; Electrical Conductivity (EC)

INTRODUCTION

Phytochemicals are those compounds which found naturally in plants. These compounds provide plant protection from many diseases. Medicinal plants are those plants in which secondary metabolites are using for treatments of many diseases. From many years traditionally medicinal plants using for health treatments in rural areas. MAPs are globally important for the treatments of many diseases and useful for the improvement in human health and prevent from cardiovascular diseases, reducing risk cancer and also play an important role in antimicrobial effect. It has been shown by many authors that medicinal plant successfully grown under hydroponic culture and which can be better in quality attributes. Growing of the crop without soil is known as hydroponics. Globally, hydroponics technology has been accepted as sustainable technology. Presently hydroponics is an emerging technology which is principally based on "Right time, right manner, and right quantity". Which play an important role in precision farming. Hydroponics not only offers safe food but also ensures vertical utilization of space with higher water use efficiency, nutrients availability, cropping intensity by using vertical space, protection from soil borne diseases, early yield and higher productivity with better quality. With increasing the urbanization, greenhouse effect, global warming and decreasing per capita land availability, unpredictable weather condition, increasing of carbon foot print and the tendency of people towards organic food these challenges bring out us towards the hydroponics cultivation. Soilless cultivation technology plays important role in the sustainable food production chain with better quality, and the boon for the cultivation of medicinal plants under indoor limited space with rich in nutraceutical properties. Soil is the natural media for crop cultivation. Himalayan seasoning *Allium* or Stracheys Chive *Allium stracheyi* is a member of Amaryllidaceae family. Vernacular name is "Fern, jambu". *Allium stracheyi* is enlisted as Vulnerable in the Red Data Book of Indian Plants and also categorized it as Vulnerable in the western Himalayan

states of Jammu & Kashmir, Himachal Pradesh, and Uttarakhand. It is found at high altitude area and commonly known as Jamboo, and Dhungar in Uttarakhand, India. It is a good source of income for the tribal communities of Uttarakhand with medicinally and economically importance ^[1]. Other culinary herb is sweet basil (*Ocimum basilicum* L.). In Hindi it is known as Babauji tulsi. Among various *Ocimum* species, *Ocimum basilicum* is commercially and extensively cultivated for essential oil production and the phyto constituent's anti-inflammatory, antioxidant found in sweet basil ^[2]. Mint is the aromatic, herbaceous plant used for menthol extraction it belongs to the family Lamiaceae. Considering that certain environmental condition and anatomical parts of plant significantly varies on secondary metabolites synthesis. The aim of present study was to determine of phytochemical compounds of different medicinal and aromatic herbs under different growing condition.

MATERIALS AND METHODS

Hydroponics system used for growing the crop under NFT structure frame unit designed and fabricated in low-cost shade net-house, the unit made of commercially available PVC pipe of 4" diameter. Two pipes of equal length were fix on the frame and provision of 4 wholes was made on each to accommodate 4 plants. Nutrient solution supplied to grow the plants in hydroponics system carried all essential nutrients with pH ranging from 6.5 to 7.5 during the experimentation and Electrical Conductivity (EC) was 1400 ppm \pm 100 ppm. Electrical Conductivity (EC) and pH of nutrient solution were checked by using hand held pH and EC meter (MCP and HANNA, respectively). Crop grown under soilless cultivation system under UV stabilized bucket with the mixture of coco peat, vermiculite and perlite in the ratio of 3:1:1 respectively. The same nutrient solution frequently applied for growing crop in hydroponics system, soilless media and soil growing condition. The crop growing in soil was sandy loam in texture, having pH 6-6.5. The life cycle of Jambu crop was biennial (secondary bulb formation) but the sample collected from fresh leaves after 75 days-80 days after transplanting under soil grown vs. hydroponically grown condition. Whereas the collection of samples from fresh leaves of sweet basil and mint from 90 days-95 days after transplanting. Data were recorded from randomly selected plant for biochemical quality attributes evaluation.

Determination of total phenols

The Folin-Ciocalteu method was used to calculate total phenols present in the plant extract ^[3]. Gallic acid was employed as the benchmark; its concentration was 5 mg/50 ml. 250 mg of fresh leaves, was weighed then crushed in a mortar-pestle and thoroughly mix 0.2 ml of (80%) ethanolic extract (4 mg/ml) and 0.2 ml of Folin-Ciocalteu reagent, 60 μ l sample was used for examination. 1 ml of 15% sodium carbonate was added after 4 minutes of incubation in dark and the mixture was left to stand at room temperature for 2 hours. At 650 nm, the absorbance was measured. Using a standard calibration curve, the concentration of total phenols was determined to be equal to gallic acid.

Determination of tannins

Total tannin in plant extract was determined by Folin-Denis method ^[4]. Fresh sample of the plants was finely crushed by using mortar and pestle and transferred into centrifuge tube and centrifuged at 2000 rpm for 20 min the supernatant was collected in 100 ml volumetric flask and the volume was made up with double distilled water. 1 ml of this solution was transferred to a 100 ml volumetric flask and diluted with 75 ml water and 5 ml of Folin-Denis reagent. 10 ml of sodium carbonate solution was added and volume was made up to 100 ml with deionized water. After shaking, the absorbance was recorded at 700 nm after 30 min. Blank solution was prepared with water

instead of the sample. Standard graph (Figure 2) was prepared by using different concentrations of 0 µg-100 µg tannic acid. Total tannin content of the sample was measured equivalent to tannic acid by standard graph [5].

Determination of ascorbic acid content

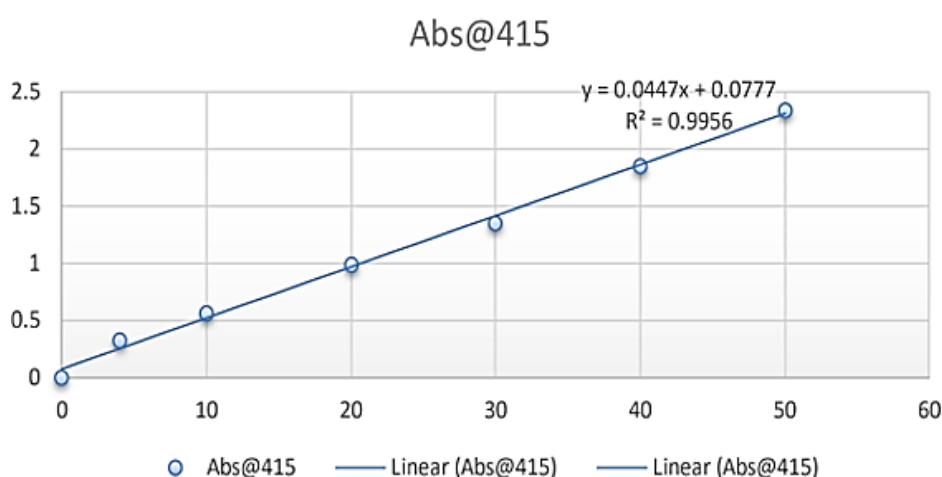
Total ascorbic acid content in plant extract was determined by 2,6-dichlorophenolindophenol method [6]. 2 g sample was extracted with 4% oxalic acid and the volume was made up to 100 ml. It was centrifuged at 10,000 rpm for 10 min. 5 ml supernatant liquid was transferred to a conical flask and 10 ml of 4% oxalic acid was added. It was titrated against standard dye solution (2,6-dichlorophenolindophenol) taking pink colour as end point. The procedure was repeated with a blank solution (without adding sample). 5 ml ascorbic acid of 100 ppm was used as standard.

Ascorbic acid content was calculated using the formula: Ascorbic acid (mg/100 g)=[0.5 mg × titer volume against test × 100 ml/titer volume against reference × 5 ml × weight of sample] × 10

Determination of total flavonoids

Total flavonoids in plant extract was determined by aluminium chloride method was modified from the procedure given by Meena et al [7]. Quercetin (Sigma) was used as standard 100 mg-250 mg of the sample was weighed and was crushed with a pestle and mortar in methanol. Then the sample was centrifuged at 10,000 rpm for 20 min and supernatant was collected. 0.5 ml of solution extract was taken and 1.5 ml of methanol, 0.1 ml of potassium acetate (1 M), 0.1 ml of aluminum chloride and 2.8 ml of distilled water were added. The reaction mixture was incubated at room temperature for 30 minutes and absorbance was measured 415 nm against the reagent blank. Blank was prepared with water instead of sample. Calibration curve was prepared by using quercetin (1 mg/ml) and standard graph was plotted against concentration and absorbance. Standard curve was used to calculate the amount of flavonoids equivalent to quercetin present in the sample as shown in Figure 1.

Figure 1. Calibration curve for flavonoid estimation.



Hydroponic nutrient solution

Hydroponics nutrient solution was standardized at DIBER on growth, yield and quality of various vegetables crops. The solution performed well in respect to crop growth, yield and quality. Keeping in the mind that nutrient solution was prepared followed by protocols that there are no chances of precipitation of nutrients. The solution consists all essential (Macro and micro) nutrients. In hydroponics solution the complex of cations (positively charged (+)) and

anions (negatively charged (-)) presence which makes the electrical conductivity concentration. According to ions properties, in this context hydroponics nutrient solution prepared, there is an antagonized between anions and cations which sometimes encourage the precipitation of nutrients. Thus, the focus is highlighting that preparation of hydroponics solutions there was three categories of stock solutions i.e., A, B, C. category A consisting KNO_3 , KH_2PO_4 , Na_2MoO_4 . Category B consisting $\text{Ca}(\text{NO}_3)_2$, H_3BO_3 , MnCl_2 , Boric acid to be dissolved in hot water then boil it after dissolved it and make the volume 1 liter which add 10 ml of this stock solution/100 liters of water. In category C, Iron stock solution prepared separately, firstly weighing (66.6 g/2 lit.) chelating agent EDTA and dissolved in 600 ml distilled water and weighing 8 g. NaOH (Pellets) dissolved in 200 ml of water than warm up to 72°C than weighing 50 g FeSO_4 and dissolved the prescribed quantity in 600 ml distilled water and add 1% N H_2SO_4 (10 ml/ 2 lit) and warm up to 75°C - 80°C , then mix both the solution and expose to aeration overnight to get ready to use (1 ml/lit) iron solution with Fe solution there are also added micro nutrients such as Mn^{2+} , Zn^{2+} , and Cu^{2+} . Our hydroponic nutrient solution considering well availability of nutrients according to their concentration and ions reactions. It is also must be worth saying that the correlation between K^+/Na^+ and $\text{Cl}^-/\text{NO}_3^-$ could represent a limiting factor for soilless cultivation of crop cultivation, especially in area where saline water is main problem [8]. Under hydroponics cultivation EC maintaining is very necessary because the excessive level of nitrates accumulates in plant leaves, excessive level of phosphorus also impacts on crop growth. Thus, the recommended dose of nutrients in solution must be necessary. For increasing the EC concentration add macro nutrients. Concentration of elements in nutrient solution used for growing crop was nitrogen (250 ppm), potassium (300 ppm), phosphorus (36 ppm), calcium (200 ppm), magnesium (60 ppm), zinc (3 ppm), manganese (3 ppm), copper (less than 1 ppm), boron (4 ppm), molybdenum (less than 1 ppm), sodium (less than 1 ppm), and Sulphur (50 ppm).

Sample preparation

Firstly, collect the sample from different growing condition. The fresh leaves of crop plant harvested and washed with tap water and crushed with the help of mottle and pestle in the methanol. The extracts were centrifuged and the supernatant was collected. An additional 20 ml of 80% ethanol were then used to extract the residual precipitate and centrifugation was carried out under the same conditions.

Statistical analysis

Statistical analysis was done by using standard software. The significance of variation among the treatments was observed by applying analysis of variance (ANOVA) and Critical Difference (C.D.) at 5% level of significance for each character was worked out.

RESULTS AND DISCUSSION

Effect on phytochemical attributes

Phytochemicals are the important constitutes which provide beneficial role in human life by protecting from many diseases. The secondary metabolites are produced in medicinal plants which play an important role in pharmaceutical uses. In phytochemical compounds phenol, Tannin and flavonoids are the important antioxidant compounds. The results (Table 1) revealed that the phytochemical quality attributes significantly differed under different growing condition. The result exhibited that's the tannin and flavonoid content in jambu crop determined higher (235.42 mg TAE/100g, 10.44 mg QE/g respectively) under hydroponic growing condition which exceeded almost 3 times to soil grown condition. Whereas the total phenol compound found higher (623.44 mg GAE/100 g) under soil growing condition as compared to other growing conditions. The result (Table 2) revealed that's the

determination of phytochemical compounds in mint aromatic plant the total phenol and flavonoid attributes found higher (632.75 mg GAE/100 g, 25.34 mg QE/g respectively) under soilless growing condition which is almost 2.5 times higher as compared to other grown condition. Whereas the tannin compound determined higher (198.49 mg TAE/100 g) under hydroponics growing condition. Similar results have also been reported in peppermint by recorded in *Mentha spicata* L. that's yield, organic acid concentration, and antioxidant content were higher in hydroponically grown plants [9,10].

The results (Table 3) revealed that's the phytochemical quality attributes in sweet basil which is tannin and flavonoid recorded 2 times higher (301.10 mg TAE/100 g, 19.00 mg QE/g respectively) under soilless growing condition as compared to other growing conditions. Whereas the total phenol content found higher (569.13 mg GAE/100 g) under soil growing as compared to other growing condition. The result (Table 4) exhibited that's the ascorbic acid content significantly differs under different growing condition. Jambu herb exhibited heigh (34.81 mg/100 g) ascorbic acid under soil growing condition as compared to other growing media.

On the other hand, the ascorbic acid found (11.09 mg/100 g, 14.63 mg/100 g, respectively) in mint and sweet basil under hydroponics growing condition as compared to other growing condition. Cultivation of medicinal and aromatic herbs under hydroponic system which leads to the higher accumulation of bioactive compounds due to the well availability and proper supply of nutrients as well as protected from soil borne diseases. Our finding is also supported by the reports [11]. Found that's cultivation of medicinal plants under hydroponics which can be given the result higher concentrations of bioactive substances because of the total control the grower can have.

Table 1. Effect of growing systems on phytochemical quality attributes of jambu crop.

Growing system	Phenol content (mg GAE /100g)	Tannin content (mg TAE/100g)	Flavonoid content (mg QE /g)
Hydroponics	245.61	235.42	10.44
Soil	623.44	75.31	4.65
C.D	28.14	4.51	0.21
SE(m) ±	6.98	1.12	0.05

Table 2. Effect of growing systems on phytochemical quality attributes of mint crop.

Growing system	Phenol content (mg GAE/100g)	Tannin content (mg TAE/100g)	Flavonoid content (mg QE/g)
Hydroponics	436.19	198.49	18.23
Soilless	632.75	39.45	25.34
Soil	253.63	144.93	10.55
C.D	18.03	9.84	1.05
SE(m) ±	5.11	2.79	0.298

Table 3. Effect of growing systems on phytochemical quality attributes sweet basil crop.

Growing system	Phenol content (mg GAE/100g)	Tannin content (mg TAE/100g)	Flavonoid content (mg QE/g)
Hydroponics	473.45	147.74	7.65
Soilless	535.29	301.1	19
Soil	569.13	294.07	7.08
C.D	0.11	10.29	0.36
SE(m) ±	0.03	2.91	0.1

Table 4. Effect of growing systems on ascorbic acid content (mg/100g) of different herbs.

Growing system	Jambu	Mint	Basil
Hydroponics	31.54	11.09	14.63
Soil	34.81	7.12	9.6
C.D	NS	1.83	0.11
SE(m) ±	2.66	0.28	0.01

CONCLUSION

Our study is concluded that phytochemical quality attributes found higher under hydroponically grown herbs as rest on the other growing conditions. This study will be beneficial where scarcity of water availability and land shortage are the main problem. This study will be also beneficial for remote area and play a vital role for human health protection from many diseases.

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