# EVALUATION ON THE GROWTH, NUTRITIONAL AND ANTI-NUTRITIONAL FACTORS IN MICROGREENS GROWN ON COCOPEAT AND VAM ASSOCIATED COCOPEAT AS SUBSTRATE

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## ABBREVIATIONS

- ABTS -2,2'-azino-bis(3-ethyl benzothiazoline-6- sulfonic acid)
- BSA -Bovine Serum Albumin
- DPPH -2,2-diphenyl-1-picryl-hydrazyl-hydrate
- FRAP -Ferric Reducing Antioxidant Power
- GAE -Gallic acid Equivalent
- $H_2SO_4$ -Sulphuric acid
- HCl -Hydrochloric acid
- $IC_{50}$ -Half-maximal inhibitory concentration
- $K_2HgI_4$ -Potassium mercuric iodide
- $Na_2CO_3$ -Sodium carbonate
- ROS -Reactive Oxygen Species
- gFW -Fresh weight in grams
- TPC - Total phenolic content
- TAC - Total antioxidant capacity
- GMC -Green mustard grown in cocopeat
- GMV - Green mustard grown in VAM
- RPC - Radish pink grown in cocopeat
- RPV -Radish pink grown in VAM
- SPC -Spinach grown in cocopeat
- SPV -Spinach grown in VAM
- SFC -Sunflower grown in cocopeat

- SFV - Sunflower grown in VAM
- WGC -Wheat grass grown in cocopeat
- WGV - Wheat grass grown in VAM

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# ABSTRACT

Microgreens are emerging ‘super foods ‘ which are gaining popularity as a new culinary ingredient. They are young and tender cotyledonary leafy greens that are found in a pleasing palette of colors, textures, and flavors. They are used to enhance salads or as edible garnishes to embellish a wide variety of other dishes. Common microgreens are grown mainly from mustard, radish, buckwheat, lettuce, spinach, etc. The consumption of microgreens has nowadays increased due to higher concentrations of bioactive components such as vitamins, minerals, and antioxidants than mature greens, which are important for human health. However, they typically have a short shelf life due to rapid product deterioration which make their prices high. Research has explored preharvest and postharvest interventions.In this study, nutritional profile, phytochemicals such as total phenolics (TP) and total flavonoids (TF) with antioxidant functions were quantified in five culinary microgreens. The antioxidant capacities of methanolic extracts of these microgreens were also determined using assays targeting different antioxidant mechanisms like DPPH . Thus, microgreens rich in antioxidant components could be used as a promising health-promoting culinary ingredient.

# CHAPTER 1

# INTRODUCTION

Microgreens, a novel form of leafy vegetables are now gaining popularity as a culinary ingredient.They are emerging edible vegetables which are approximately 1-3 inches tall gaining popularity in the twentyfirst century. They are considered as baby plants falling between sprouts and baby leaf vegetables. They are young and tender cotyledonary leafy greens available in pleasing palette of colours, texture and flavours which are rich in concentrated bioactive compounds such as vitamins, minerals, antioxidants etc which are important for human health compared to mature leafy greens. Beyond the function of supplying nutrients, they contain health promoting or disease preventing properties for which referred to as 'functional foods' (Xiao *et al.,* 2014).

These aromatic greens also known as micro herbs or vegetable confetti are harvested when first leaves have fully expanded and before true leaves have emerged, usually 7-21 days after germination.They are very convenient to grow in a variety of locations including outdoors,greenhouses and even in windowsills. They can be grown from different types of seeds. Most popular varieties are from the seeds of families like Brassicaseae family (cauliflower, broccoli, cabbage, radish etc), Asteraceae (Lettuce, chicory etc ), Apiaceae (carrot, celery, fennel etc), Amaryllidaceae (garlic,onion), Amaranthaceae (beet, spinach etc ), Cucurbitaceae( melon, cucumber, squash).Their taste varies depending on their variety.

Microgreens are packed with nutrients. They tend to be rich in potassium, iron, copper, zinc and magnesium. They are a great source of beneficial plant compounds like antioxidants and polyphenols much more than their mature counterparts.

Microgreens have the ability to lower the risk of heart disease and alzheimer's disease as they contain rich source of polyphenols. They reduce type of stress that prevent sugar from entering the cells thereby helping diabetic persons. Antioxidant rich fruits and vegetables especially those rich in polyphenols may lower the risk of various types of cancer.

Eating microgreens is generally considered safe.The potential for bacterial growth is much smaller in microgreens than in sprouts.Microgreens require slightly less warm and humid conditions than the sprouts do and only the leaf and stem rather than root and seed are consumed. The most common growing mediums are peat, perlite and vermiculite.

However, microgreen consumption is limited by their low productivity, rapid senescence and a very short shelf life, usually 3–5 d at ambient temperature (Kou *et al*., 2014).

The vegetable species used in microgreen production belong to several botanical families, such as Asteraceae, Apiaceae, Amarillydaceae, Amaranthaceae, Brassicaceae, Cucurbitaceae, and Fabaceae, for which several different phytochemicals with antioxidant and healthy properties have been reported. Most of the published studies involved Fabaceae and Brassicaceae [Mir *et al*.,2017]; microgreens belonging to the latter family, in particular, were reported as good sources of K, Ca, Fe and Zn [Xiao *et al.,*2016]. The phytochemical composition of Brassicaceae varies considerably as a consequence of the plant growth stage and the analyzed species [Scialabba *et al*.,2010].

In the present study, microgreens of five species were analyzed: Green mustard, Radish pink,Spinach,Sunflower and Wheat grass.. For each species, selected phytochemical compounds with nutritional value (chlorophylls, polyphenols, carotenoids, anthocyanins, ascorbic acid, total and reduced sugars), their antioxidant activity were evaluated. The antioxidant capacities of

methanolic extracts of these microgreens were also determined using assays targeting different antioxidant mechanisms like DPPH .

The study included evaluation of seed germination and growth pattern for two weeks period and nutritional characterization of selected microgreens. Results showed that the seeds of selected plants had > 80% germination and attained 6–7 cm height within two weeks. Microgreens had low carbohydrates and anti-nutrient contents; but high vitamin contents, so these can be recommended as dietary supplement, especially for those who preferred less carbohydrate-containing food supplements.(Nair and Lakshmi.,2019)

The abundance of bioactive compounds in microgreens, including vitamins, minerals, and phytochemicals, has been examined in many research studies. Researchers have been particularly interested in analyzing antioxidants that neutralize free radicals and help prevent damage caused by oxidative stress, such as vitamin C (VC), phytochemicals (e.g., carotenoids and phenolics), and certain minerals, including copper (Cu), zinc (Zn), and selenium (Se). There have been comparisons of antioxidant contents and capacity between microgreens and their mature counterparts [E Pinto *et al*.,2015]. Several microgreens showed higher concentrations of antioxidants, but the results were not generalizable [Yadav *et al*.,2019].

Several trace minerals, i.e., Cu, Zn and Se, as cofactors or components of antioxidant enzyme (such as superoxidase dismutase), play an essential role in the endogenous antioxidant defense system of human body, and are therefore referred to as antioxidant minerals. Inadequate intake of antioxidant minerals in the diet can reduce the activity of antioxidant enzyme [Harris, 1992]. These antioxidant minerals, among other minerals, have been routinely analyzed in microgreen samples and compared with their mature plants [Lenzi *et al*.,2019)

Phytochemicals, such as carotenoids and phenolics, are also found in abundance in microgreens. Carotenoids are a group of lipophilic plant pigments showing yellow, orange, and red color, including carotenes (e.g., β-carotene and lycopene) and xanthophylls (e.g., lutein and zeaxanthin) . Carotenoids possess antioxidant activity and play important physiological roles in human body [Rodrigues and Amaya.,2015]. Vegetables, especially bright colored ones, can be major dietary sources of carotenoids [Khoo *et al*.,2011].

Phenolic compounds are the most abundant secondary metabolites of plants ranging from small molecules, e.g., phenolic acids, to flavonoids with multiple rings, and to highly polymerized compounds, e.g., tannins . Phenolics are antioxidants for plants to repair damage caused by free radicals and have shown many health benefits for human [Dai and Mumper.,2010]. Sun *et al.* [Sun *et al.*,2013] identified 164 polyphenols, including 30 anthocyanins, 105 flavanol glycosides, and 29 hydroxycinnamic acids, in the 5 Brassica species microgreens. Microgreens have more complex polyphenol profiles and higher contents than mature Brassica plants [Cartea *et al*.,2011], making them good sources of antioxidants.

# CHAPTER 2

# AIM AND OBJECTIVES

The present study focused on the production of microgreens, an emerging culinary ingredient popular for their potentially high content in nutraceuticals and their nutritional profile.The aim was to assess the individual characteristics of each species of microgreens taken, in order to obtain a more complete overview of their potential nutritional value.

The main objectives of the study are as follows :

- Production of microgreen  and their extraction.
- Determination of moisture content of microgreens
- To analyse and evaluate microgreens for  antinutritional and nutritional  content.
- Determination of antioxidant activity

# CHAPTER 3

# REVIEW OF LITERATURE

Over the past twenty years, the heavy dependence of human nutrition on the sustainability of agricultural production has been highlighted owing to rapid population growth and environmental degradation [Jones *et al*.,2017]. Increasing food and agricultural productivity has simultaneously imposed external expenses and consequences upon the financial resource, ecological system, and human health by excessive water use, soil occupation, fertilizers, pesticides, herbicides, and food waste treatment [Weber,2017]. Inorder to reach the sustainable development goals, the agriculture and food sectors have been confronted with a major challenge to provide adequate nutrition for global food demand while minimizing the negative impacts on the environment .

Furthermore, in recent years, improving public awareness about the healthy lifestyle framework has prompted the search for novel food sources, which are rich in essential nutrients and have positive effects on human health.As a result, functional foods and nutraceuticals are gaining significant attention since these foods could provide both health benefits to reduce the risk of chronic diseases and basic nutrition [Gupta *et al*,2013]. Particularly, the issues of the food systems, including food safety, food security, and food sustainability, should be significantly addressed in the era of the coronavirus (COVID-19) pandemic crisis. The availability of bioactive constituent of food and functional foods may become essential as the demand of consumers to protect their immune system by applying healthier diets increases. The food industry should have innovations fast enough in the era of the COVID-19 pandemic crisis to offer functional foods fortified with bioactive compounds that promote health and support consumers' immune system . Thus, emerging technologies have been considerably studied and

developed for the production of functional compounds from food sources, comprising extraction, separation, isolation, identification, and quantification [Galanakis,2020]. Sprouts and microgreens are a new approach for functional foods with various advantages from the sustainability perspective, inclusive of eliminating the use of herbicides and pesticides, reducing the generation of food waste, and reaching a 10-fold increase of health-promoting phytochemicals compared to commercial adult plants [Baenas *et al*.,2012].

Various types of young seedlings of vegetables and herbs, including sprouts, microgreens, and baby greens, are becoming popular due to their production technique and nutritional value [Mir S.A *et al*.,2017]. Sprouts are distinct from microgreens, even though both products are consumed in an immature stage . Sprouts constitute shoots and rootlets, obtained from germinated seeds that grow for 2–7 days, and are harvested when the cotyledons are still under developed and the true leaves have not yet emerged. Different to sprouts, microgreens are typically recognized by the full expansion of the cotyledon leaves and the appearance of the first true leaves that generally occurs within 7–21 days after sowing [Kyriacou *et al*.,2017]. Sprouts and microgreens are ideally appropriate for indoor production with significant improvement of the safety properties, which obviates the presence of external contaminants, such as herbicides, pesticides, or heavy metals . Additionally, seedling cultivation is a flexible and qualified process without the unwanted impacts of seasonal, climatic, and geographical variations [Turner E.R *et al*.,2020].

The Brassicaceae family, consisting of plants with an acrid taste (commonly known as Cruciferae), in general, and broccoli (*Brassica oleracea* L. var. *Italica*), in particular, has received considerable interest for the production of sprouts and microgreens [Baenas *et al* .,2017]. Available data revealed that the consumption of broccoli sprouts and microgreens in a dietary serving plays an important role in human health and reduces the risk of chronic diseases.

Numerous in vitro and in vivo studies indicated the chemical composition and multiple biological capacities of broccoli sprouts and microgreens, comprising antioxidant, anticarcinogenic, antimicrobial, anti-inflammatory, and antidiabetic activities [Lu *et al*.,2018]. The anticancer and antioxidant properties in particular have been comprehensively studied over recent years. Furthermore, human-based investigations have presented promising results about broccoli seedlings' potential as a protective agent for several forms of cancer and other diseases [Bahadoran *et al*.,2012].

Bioactive compounds are extra-nutritional constituents found in foods, mainly in fruits, vegetables, and grains, which are capable of modulating metabolic processes and providing health-promoting benefits [Santos *et al*.,2019].Thus, they are considered a novel plant-derived functional food. Previous studies have extensively indicated that microgreens contain a remarkably high amount of glucosinolates, phenolic compounds, and essential nutrients. There are numerous methods employed for the extraction of bioactive compounds from herbs, vegetables, and fruits, including emerging technologies such as pressurized hot water extraction, microwave-assisted extraction, or pulsed electric extraction [Barba *et al*.,2017]. The separation, identification, and characterization of these compounds seedlings were investigated by both conventional and non-conventional technologies. Among these technologies, samples were mainly conducted by high-performance liquid chromatography (HPLC) coupled to diode array (DAD), ultraviolet-visible (UV–vis), electrospray ionization (ESI), or mass spectrometry (MS) detectors with $C_{18}$ analytical columns. Gas chromatography combined with mass spectrometry (GC–MS) was also applied to characterize isothiocyanates, the hydrolysis products of glucosinolates. Besides, the spectrophotometric (UV–vis) detection was employed in most of the research to determine the total phenolic and flavonoid contents as the simplest procedure, if it

was not required to determine a specific compound . It could be summarized that glucosinolates and related compounds are the major group of phytochemicals investigated in broccoli sprouts and microgreens, although phenolic compounds have also been analyzed in many studies.

Another important group of bioactive constituents present in cruciferous vegetables is the phenolic compounds. They are secondary metabolites produced in plants through the phenylpropanoid and shikimate pathways [Kumar.N *et al*.,2019]. Based on their structure, which comprises one or more aromatic rings with attached hydroxyl substituents, phenolic compounds can be categorized into various subgroups, such as phenolic acids, flavonoids, tannins, coumarins, lignans, quinones, stilbenes, and curcuminoids. These compounds have been mainly reported for antioxidant activity. Moreover, they are also associated with other health-promoting effects such as anticarcinogenic, antimicrobial, anti-inflammatory, and anti-aging properties [Kumar.N *et al*.,2019].

The overproduction or incorporation of free radicals, such as reactive oxygen species (ROS), cause oxidative damage to biomolecules and consequently lead to many chronic diseases, including neurodegenerative diseases, cardiovascular diseases, and certain age-related cancers [Uttara.B *et al*.,2009]. ROS are previously thought to form in mammalian cells almost exclusively as a consequence of mitochondrial metabolism. Recently, it has been demonstrated that cellular enzymes known as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases produce a considerable amount of ROS in the human body. Moreover, other cellular sources of ROS involve neutrophils, monocytes, endothelial cells, xanthine oxidases, cytochrome P450, lipoxygenases, and nitric oxide synthesis [Bardaweel S.K *et al*.,2009]. Hence, ROS has distinct effects on normal physiological processes, oxidative stress/regulation, metabolic diseases, and chronic inflammation. Targeting ROS is involved in antioxidant, anti-inflammatory, antidiabetic,

and anti-obesity therapeutics [Alfadda_*et al*.,2012]. Antioxidants are divided into natural and synthetic compounds, which could remove free radicals, inhibit ROS formation, and scavenge ROS [Uttara.B *et al*.,2009]. Previous studies on broccoli sprouts and microgreens have demonstrated that they are recognized for their variety of naturally occurring antioxidants, comprising both nutritional antioxidants like vitamins (especially, ascorbic acid and α-tocopherol), and non-nutritional antioxidants such as carotenoids and phenolic compounds [Jang *et al.,*2015]. It was reported that these compounds are responsible for 80−95% of the total antioxidant capacity in broccoli sprouts [Baenas *et al*.,2012].

Among the possible methods, DPPH radical scavenging assay, ABTS radical cation decolonization assay, and ferric reducing antioxidant power (FRAP) assay were commonly employed in previous studies. The DPPH method is speedy, simple, and low cost in comparison to other test models. DPPH, a dark crystalline molecule, is a stable chromogen radical formed by the delocalization of the spare electron over the molecule. The DPPH scavenging assay is based on the electron donation of antioxidants to neutralize DPPH radical. The reaction occurs with the loss of the violet color of DPPH that is measured at 517 nm, and the discoloration acts as an indicator of the antioxidant effectiveness [Ares A.M *et al*.,2013]. The ABTS decolonization assay is appropriate for both hydrophilic and lipophilic antioxidants. It uses a spectrophotometer to measure the ability of antioxidants to scavenge the stable radical cation $ABTS^{+}$, a blue-green chromophore molecule with absorption at 734 nm. Antioxidants can neutralize and decolorize the radical cation $ABTS^{+}$ by electron or hydrogen atom donations [Shahidi.F *et al*.,2015]. The FRAP assay was originally employed to measure reducing power in plasma, but it has been expanded for other biological fluids and plant extracts. Thus, it is applicable for both in vitro and in vivo experiments. The FRAP mechanism is based on electron transfer rather than hydrogen

atom transfer. The assay demonstrates the ability of antioxidants to reduce ferric iron. It measures the reduction of the ferric ion ($Fe^{3+}$)–ligand complex to the blue-colored ferrous ($Fe^{2+}$) form at low pH by antioxidants (absorption at 593 nm) [Alam M.N *et al.*,2013].

Polyphenols, including their subcategory, flavonoids, are ubiquitous in all plants. Polyphenols traditionally have been considered antinutrients by animal nutritionists, because of the adverse effect of tannins, one type of polyphenol, on protein digestibility (Bravo, 1998). However, recent interest in food phenolics has increased greatly, owing to their antioxidant capacity (free radical scavenging and metal chelating activities) and their possible beneficial implications in human health. Laboratory studies have shown that specific flavonoids suppress tumor growth, interfere with sexual hormones, prevent blood clots, and have anti-inflammatory properties. Among the important flavonoids are resveratrol, quercetin, and catechin. Evidence suggests that resveratrol (found in red wine, grapes, olive oil) may be extremely potent. In laboratory studies, it increases cell survival and has been shown to increase the life span of worms and fruit flies. Catechins are the primary flavonoids in tea and may be responsible for its possible beneficial effects. Flavonoids in dark chocolate may also be health protective (Kris-Etherton *et al.*, 2002).

## 3.1 HARVESTING MICROGREENS

Microgreens were harvested 7 days (C) or 14 days (HF and HW) after sowing using ethanol-cleaned scissors to cut the stems as close to the growth substrate as possible. Microgreens were weighed immediately on an analytical balance to determine the total fresh weight in grams (gfw). From each experimental replicate, ca. 0.1 gfw was placed into a protein extraction filter cartridge (see "Protein analysis," below), and 0.150 to 0.230 gfw were placed

into 10 mL conical tubes containing 5 mL of sterile 1X phosphate buffer (see Appendix) for washing microbes from the microgreen surfaces to determine microbial counts. The remaining biomass was weighed and placed into a drying oven at 80°C for 48 hours, after which the microgreens were weighed again to determine their water content.

## 3.2 POSTHARVEST STORAGE

Storage temperature is one of the most important factors affecting the postharvest physiology and storage behavior of produce. In general, low temperature storage can reduce quality loss and extend shelf life by depressing rates of respiration, senescence, and growth of spoilage microorganisms (Spinardi & Ferrante, 2012). Optimum storage temperature varies depending on the fruit or vegetable. For some chilling sensitive fruits and vegetables, the use of low temperature storage adversely affects quality attributes and causes deterioration more rapidly .Even though an optimal low temperature is maintained through the storage, transportation and retail, the fruits and vegetables can still spoil, as evidenced by fungal attacks and detrimental quality changes. Therefore, low temperature storage should be combined with other postharvest handling methods, like modified atmosphere packaging or UV irradiation. (Xiao *et al*.,2014)

## 3.3 PRODUCTION

Microgreens may be grown by individuals for home use. Growing small quantities at home is relatively easy; however, growing and marketing high-quality microgreens commercially is much more difficult. Having the right mix at the perfect stage for harvest is one of the most critical production strategies for success. The time from seeding to harvest varies greatly from crop to crop. When seeding a mixture of crops in a single planting flat, growers should select

crops that have a similar growth rate so the entire flat can be harvested at once. Alternatively, growers can seed the various crops singularly and mix them after harvest.

Microgreens can be grown in standard, sterile, loose, soilless germinating media. Many mixes have been used successfully with peat, vermiculite, perlite, coconut fiber, and others. Partially fill a tray with the medium of choice to a depth of ½ in. to 1 or 2 in., depending on irrigation programs. Overhead mist irrigation is generally used only through the germination stage in these media systems. After germination, trays should be subirrigated to avoid excess moisture in the plant canopy.

An alternative production system uses one of several materials as a mat or lining to be placed in the bottom of a tray or longer trough. These materials are generally fiberlike and provide an excellent seeding bed. Materials may include burlap or a food-grade plastic specifically designed for microgreens, such as those made by Sure to Grow (Beachwood, OH). These mat systems are often used in a commercially available production system using wide NFT-type troughs. The burlap mat may be sufficient alone for certain crops or may require a light topping with a medium after seeding. Seeding may be done as a broadcast or in rows. Seeding density is difficult to recommend. Most growers indicate they want to seed as thickly as possible to maximize production, but not too thickly because crowding encourages elongated stems and increases the risk of disease. Most crops require little or no fertilizer, because the seed provides adequate nutrition for the young crop. Some longer-growing microgreen crops, such as micro carrot, dill, and celery, may benefit from a light fertilization applied to the tray bottom. Some of the faster-growing greens, such as mustard cress and chard, may also benefit from a light fertilization because they germinate quickly and exhaust their self-contained nutrient supply

quickly. Light fertilization is best achieved by floating each tray of microgreens for 30 seconds in a prepared nutrient solution of approximately 80 ppm nitrogen. (Treadwell *et al*.,2010)

Microgreens are ready for harvest when they reach the first true leaf stage, usually at about 2 in. tall. Time from seeding to harvest can vary greatly by crop from 7 to 21 days. Production in small trays will likely require harvesting with scissors. This is a very time-consuming part of the production cycle and is often mentioned by growers as a major drawback. The seeding mat type of production system has gained popularity with many growers because it facilitates faster harvesting. The mats can be picked up by hand and held vertically while an electric knife or trimmer is used for harvesting, allowing cut microgreens to fall from the mat into a clean harvest container. Harvested microgreens are highly perishable and should be washed and cooled as quickly as possible. To improve quality, some chefs ask growers to deliver microgreens in the trays or mats so that they can cut the microgreens as needed. Wash the microgreens using good handling practices for food safety. Microgreens are usually packed in small, plastic clamshell packages and cooled to recommended temperatures for the crops in the mix.

### 3.4 ANALYSIS OF GROWTH OF MICROGREENS

As per the analysis of growth of different microgreens cultivated in different medium, it was observed that microgreens cultivated in coco pith showed maximum harvest and minimum days for cultivation than in soil and water. Thirty grams of seeds of each microgreens variety were used for cultivation. It was observed that the number of days of cultivation of fenugreek, amaranth, spinach, mint and fennel microgreensin soil were observed as 9, 10, 12, 12 and 18 days respectively,  coco pith were observed as 8, 8, 9, 10 and 18 days respectively. The growth pattern of the microgreens was observed to be ranging from 3.5 to 7.0 cm at the time of harvest.

From the microgreens cultivated both in soil and coco pith, fennel microgreens were observed to have the highest length i.e. 7.0 cm and amaranth microgreens were observed to have the shortest length i.e. 4.6 cm. From the microgreens cultivated in water, it was observed that both fenugreek and amaranth microgreens were observed to have the length of 3.5 cm. Failure in cultivation of spinach, mint end fennel microgreens were observed due to rotting of roots and improper cultivation techniques.The yield of the microgreens were observed to range from 30 to 150 gm at the time of harvest. Coco pith cultivation was observed to have higher yield than soil and water. It was observed that fenugreek microgreens had the highest yield of 150gm and fennel microgreens had the lowest yield of 50 gm. ( M Sinha. 2021)

### 3.5 VAM ASSOCIATED STUDY IN MICROGREENS

VAM (Vesicular arbuscular mycorhizae) are perhaps the single most important group of soil microorganisms because they play an important role in a number of key soil health functions, such as nutrient cycling, plant water relations, plant disease resistance and soil aggregation. It is formed by the symbiotic association between certain phycomycetous fungi and angiosperm roots. VAM colonisation of plant roots may start from a single spore and, under ideal conditions, spread rapidly throughout the root system of the host plant, providing an efficient extension of its root system and increasing nutrient uptake (particularly phosphorus and other nutrients that are relatively immobile in the soil. It helps to increase the immune power of plants. The presence of VAM generally enhances the nitrogen fixation process. The major benefits of VAM to the plant are the supply of inorganic nutrients as well as enhanced water absorption. Phosphate which is mostly present in the unavailable form in the soil, becomes abundantly available to the plant. Besides, the fungus provides various growth promoting

substances.The vast majority of plant species used in urban horticulture will benefit from the presence of mycorrhizal fungi. A few examples of plants that have responded well to mycorrhizal inoculants are as follows

- Urban vegetable crops in soil or trays: onion, garlic, carrots, potatoes, tomatoes, peppers, cucurbits, asparagus, herbs and lettuce
- Annuals in planters or flower beds: salvia, ornamental grasses, canna, ferns, aloe, gerbera
- Perennials in containers or flower beds: hosta, rose bush, lavender, thyme, purple coneflower, beebalm and nepeta
- Trees and shrubs in the landscape: fruit trees, gleditsia, juniper, thujas, maples, lilacs and elms

The effects of mycorrhizae are important especially during stress episodes experienced by the plant. The greater the stress, the more the plant will rely on the mycorrhizae's cultural help and the more the plant benefits. It contributes to greater plant vigour by showing longer stem, increased plant height and width and better leaf quality.( September 24, 2021 | Troy Buechel)

### 3.6 COMPARITIVE STUDY OF MOISTURE IN MICROGREENS

According to the comparative study of the moisture content of the cultivated microgreens in different medium, it was observed that out of all the microgreens in the study, spinach microgreens were observed to have the highest percentage of moisture with 90% and mint microgreens were observed to have the lowest percentage of moisture with 84%.(M sinha, 2021)

## 3.7.TOTAL CARBOHYDRATES IN MICROGREENS

Among many colorimetric methods for carbohydrate analysis, the phenol-sulfuric acid method is the easiest and most reliable method. It has been used for measuring neutral sugars in oligosaccharides, proteoglycans, glycoproteins, and glycolipids. This method is used widely because of its sensitivity and simplicity. In its original form, it required 50-450 nmol of monosaccharides or equivalent for analysis and thus is inadequate for precious samples. A scaled-down version requiring only 10-80 nmol of sugars was reported previously. We have now modified and optimized this method to use 96-well microplates for high throughput, to gain greater sensitivity, and to economize the reagents. This modified and optimized method allows longer linear range (1-150 nmol for Man) and excellent sensitivity. Moreover, our method is more convenient, requiring neither shaking nor covering, and takes less than 15 min to complete. The speed and simplicity of this method would make it most suitable for analyses of large numbers of samples such as chromatographic fractions.( Masuko *et al*.,2005)

## 3.8.PROTEIN ESTIMATION BY LOWRY METHOD

Protein standard stock solution (1000 µg/mL) was prepared using bovine serum albumin/egg albumin 0.1 g (dry weight), dissolved in distilled water, and diluted with distilled water (100 mL). The sample dilution was carried out for calibration purposes. The method linearity was studied between 0.2 µg/mL and 1.0 µg/mL. Concentrations were converted to percent values and applied accordingly.The absorbance of the calibration solutions (0.2–1.0 µg/mL) and samples was measured at the wavelength (λmax) of 660 nm using a spectrophotometer.This research was

based on the protein content in sprouts. The λmax of the calibration solutions was found to be 0.074–0.275 nm, whereas the λmax values of the studied samples of mung beans, lentils, and chickpeas were 0.159, 0.221, and 0.110 nm, respectively. The standards and samples were analyzed in six replicates ($n = 6$). The amounts of protein in mung beans (2.96%), lentils (4.10%), and chickpeas (1.60%) were obtained. Relatively, mung beans and lentils produce higher amounts of protein than chickpeas.( Rizvi *et al.,*2022)

### 3.9.ANALYSIS OF LIPIDS IN MICROGREENS

Microgreens are progressively studied for their potentially high content in nutraceuticals, like polyphenolic compounds, carotenoids, and glucosinolates, also in the perspective of implementing their cultivation in space stations/colonies. Among further potential nutraceuticals of microgreens, lipids have received very limited attention so far. Here, glycerophospholipids contained in microgreens of typical oleaginous plants, namely, soybean, chia, flax, sunflower, and rapeseed, were studied using hydrophilic interaction liquid chromatography (HILIC), coupled to high-resolution Fourier transform mass spectrometry (FTMS) or low-resolution collisionally induced dissociation tandem mass spectrometry (CID-$MS^2$) with electrospray ionization (ESI). Specifically, this approach was employed to obtain qualitative and quantitative profiling of the four main classes of glycerophospholipids (GPL) found in the five microgreens, *i.e.*, phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylglycerols (PG), and phosphatidylinositols (PI). Saturated chains with 16 and 18 carbon atoms and unsaturated 18:$X$ (with $X = 1–3$) chains emerged as the most common fatty acyl substituents of those GPL; a characteristic 16:1 chain (including a C═C bond between carbon atoms 3 and 4) was also found in some PG species. Among polyunsaturated acyl chains,

the 18:3 one, likely referred mainly to α-linolenic acid, exhibited a relevant incidence, with the highest estimated amount (corresponding to 160 mg per 100 g of lyophilized vegetal tissue) found for chia. This outcome opens interesting perspectives for the use of oleaginous microgreens as additional sources of essential fatty acids, especially in vegetarian/vegan diets.(Castellaneta *et al*.,2022)

### 3.10. QUALITATIVE PHYTONUTRIENT ANALYSIS IN MICROGREENS

Phytonutrients like phenol, saponins, steroids, tannins and terpenoids were analysed in microgreens.It is inferred that, presence of phenols, saponins and tannins was observed in the five microgreens i.e., fenugreek, amaranth, spinach, fennel and mint. Ferric Chloride test was used to determine the presence of phenols in the sample. The fenugreek and fennel microgreens showed the presence of steroids and terpenoids. Mint microgreens showed the presence of terpenoids.(M sinha 2021)

### 3.11 TOTAL PHENOLIC CONTENT

The total phenolic concentration in aqueous extracts was determined according to the Folin-Ciocalteu method (25) using gallic acid as the standard. Four hundred microliter aqueous solutions of gallic acid and 1.6 mL of sodium carbonate (7.5% in deionized water) were added to 2 mL of Folin-Ciocalteu reagent (diluted 10-fold in deionized water). Four hundred microliter aqueous solutions of plant extract were mixed with the same reagents as described above. After incubation for 1 h at room temperature, the absorbance was measured at 765 nm. All determinations were carried out in triplicate, and the results are expressed as mg gallic acid equivalent (GAE) /g of extract. (Scalbert *et al*.,2005)

The total phenolic content was determined according to the method by ethanolic extraction following Singleton and Rossi (1965) [Singleton *et al*.,1965]. Briefly, 1 g of sample was homogenized with 15 mL of 80% ethanol and centrifuged at 12,000× *g* for 20 min. Then, 0.5 mL of filtrate was mixed with 2.5 mL of 0.2 N Folin–Ciocalteu reagent solution and 2 mL of 7.5% sodium carbonate. The mixture was incubated at RT for 90 min, and absorbance was read at 760 nm. The result was expressed as mg gallic acid equivalent (GAE) 100 $g^{-1}$ of fresh weight.

### 3.12 TOTAL FLAVANOID CONTENT

The aluminum chloride colorimetric method was modified from the procedure reported by (Woisky and Salatino,1998). Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 80% ethanol and then diluted to 25, 50 and 100 µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu UV-160A spectrophotometer (Kyoto, Japan). The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 mL of ethanol extracts or 15 flavonoid standard solutions (100 ppm) were reacted with aluminum chloride for determination of flavonoid content as described above.

The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride forms acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids(19). (Wang *et al*.,2010)

### 3.13.TOTAL ANTIOXIDANT ACTIVITY

The DPPH radical scavenging capacity of each extract was determined according to the method of BrandWilliams modified by Miliauskas . DPPH radicals have an absorption maximum at 515 nm, which disappears with reduction by an antioxidant compound. The DPPH• solution in methanol (6 × 10-5 M) was prepared daily, and 3 mL of this solution was mixed with 100 µL of methanolic solutions of plant extracts. The samples were incubated for 20 min at 37 °C in a water bath, and then the decrease in absorbance at 515 nm was measured (AE). A blank sample containing 100 µL of methanol in the DPPH• solution was prepared daily, and its absorbance was measured (AB). The experiment was carried out in triplicate. (Li *et al*.,2008)

The total antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was adapted from the method by Shimada et al. (1992) [Shimada *et al*.,1992]. One gram of the samples was homogenized with 15 mL of 80% ethanol and centrifuged for 20 min at 12,000× *g*. An aliquot of filtrate (0.1 mL) was then mixed with 2.9 mL of 0.1 mM DPPH working solution. The mixture was vortexed and incubated in the dark for 30 min at RT. The absorbance was read at 515 nm, and the result was expressed as mM Trolox equivalent antioxidant capacity (TEAC) $g^{-1}$ of fresh weight.

### 3.14 BIOACTIVE COMPOUNDS

The coloration of microgreens is one of the main attributes that affect customers' choice of microgreens and their economic value. Chlorophyll and carotenoids are major photosynthetic pigments responsible for the specific coloration of microgreens [znidarcic *et al*.,2011]. These pigments are found to be richer in microgreens than sprouts. In the present study, the

microgreens showed a total chlorophyll content range of 12.35 to 112.62 mg 100 $g^{-1}$. The smallest concentration was found in green pea, while the highest was detected in lentil microgreens. Our results bear a close resemblance to a previous study of radish and fenugreek microgreens . The content of carotenoids had a similar pattern as the content of the total chlorophyll, ranging from 4.40 to 28.37 mg 100 $g^{-1}$. Higher carotenoid contents were detected in the Brassicaceae microgreens: broccoli, Chinese kale, radish, and red cabbage, than previously reported (11.9, 10.6, 11.4, and 10.4 mg 100 $g^{-1}$, respectively) by( Xiao *et al*., 2019) . A similar carotenoid concentration (13.8 mg 100 $g^{-1}$) had been reported for purple radish . Anthocyanin pigments give the attractive red, orange, blue, or purple coloration to plant tissues . Red cabbage and purple radish exhibit purplish-red hypocotyls owing to the accumulation of anthocyanins. The total anthocyanin content detected in red cabbage was higher than purple radish. These pigments not only contribute to the visual quality of microgreens but also their biological activity, making them beneficial to human health [Ghoora *et al*.,2020].

The intake of dietary antioxidants is commonly linked with lower risks of certain serious illnesses, including cardiovascular diseases, hypertension, and diabetes [Prior *et al*.,2000]. Data from biochemical, clinical, and epidemiological research have recommended a dietary ascorbic acid intake of 90–100 mg $day^{-1}$ to lower the risks of these diseases. In the present study, the ascorbic acid detected in the microgreens ranged from 6.48 to 128.70 mg 100 $g^{-1}$. Broccoli, Chinese kale, purple radish, red cabbage, and lentil microgreens exhibited significantly higher ascorbic acid compared to the other microgreens, whereas black sesame microgreens had the least.

Phenolic compounds, products of the phenylpropanoid pathway, are one of the largest secondary metabolites primarily found in fruits and vegetables . These compounds comprise cinnamic acid, benzoic acid, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans and lignin . A significant variation in the total phenolic content of the microgreens was observed, with a range of 9.22 to 268.99 mg GAE 100 $g^{-1}$. The highest content was found in buckwheat, and the least amount was in morning glory microgreens. The detected values for radish, fenugreek, and roselle microgreens were higher than values reported by Ghoora *et al*. (2020) but lower than values for broccoli, red cabbage, Chinese kale, radish, and purple radish microgreens reported by another study. These variations could be due to various intrinsic and extrinsic factors, such as species, growth conditions, maturity at harvest, and postharvest conditions The phenolic compounds exhibit direct and indirect antioxidant actions that are beneficial to human health. Their strong antioxidant power lies in their ability to donate electrons to oxidant species, scavenge free radicals, chelate metal ions, and indirectly attenuate the accumulation of reactive oxygen species (ROS) by either improving the activity of antioxidant enzymes or inhibiting enzymes that stimulate pro-oxidant effects [Hollman,2001].

Here, the antioxidant activity of the microgreens was estimated by using DPPH• scavenging activity. Antioxidants in the microgreen extract scavenge the DPPH• through donating a hydrogen atom and converting the radical to a reduced form .The radical scavenging potential is signified by the degree of discoloration of the purple DPPH working solution. Buckwheat microgreens showed remarkably high antioxidant activity compared to others. In contrast, morning glory microgreens registered the lowest DPPH• scavenging activity. Buckwheat microgreens exhibited about 9-fold higher DPPH• scavenging activity than morning glory microgreens. It should also be noted that buckwheat and morning glory recorded the highest and

lowest TPC, respectively. Other studies have also demonstrated a strong positive correlation between TPC and DPPH• scavenging activity(Kowitcharoeon *et al*.,2021)

## 3.15.BIOCHEMICAL ANALYSIS

The aerial part of the fresh seedlings (200 mg) was used for the determination of the pigments (chlorophylls and carotenoids) by extraction in 100% methanol, following the known literature [Lichtenthaler,1987]. Fresh samples (200 mg) were extracted by homogenization in 2 mL 70% (*v/v*) methanol. After 30 min of incubation at 4 °C, the extracts were centrifuged at the maximum speed for 10 min, and the supernatants were also used for the determination of the total polyphenols by the Folin–Ciocalteau method, and the determination of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) antiradical activity, according to the published protocols [Marchioni *et al*.,2019], using an Ultraviolet-Visible (UV-VIS) spectrophotometer (SHIMADZU UV-1800, Shimadzu Corp., Kyoto, Japan). The DPPH activity was determined as $IC_{50}$ (mg/mL), namely the extract concentration required to obtain 50% of antioxidant activity. The extracts were also tested with a Ferric ion Reducing Antioxidant Power (FRAP) antioxidant assay in order to confirm the antioxidant activity [Szollosi,2002]. The total polyphenols content (TPC) was expressed as mg gallic acid equivalents (GAE) per g of fresh weight (FW). The anthocyanins were extracted from 0.1 g fresh leaves in ethanol/HCl (99/1, *v/v*), and were used to read the absorbance at 535 nm [Marchioni *et al*.,2021].

The reduced and total ascorbic acid (AsA and $AsA_{TOT}$) were quantified according to the method of Kampfenkel et al. [Kampfenkel *et al*.,1995]. Fresh samples (200 mg) were extracted in 2 mL

6% (*w/v*) trichloroacetic acid (TCA) solution, as described by Degl'Innocenti et al. [Degl'Innocenti *et al*.,2005]. The data are reported as μg of $AsA_{TOT}$ per g of FW.

The highest water content (%) was found in white radish (93.59%), followed by rocket salad (93.31%), broccoli (91.06%), watercress (88.94%), and finally mustard (86.75%). In correlation with the water content (inversely proportional), the percentage of the dry weight was quite high in the case of mustard and watercress, followed by broccoli; the lowest values were measured for rocket and daikon.

The highest reducing sugar content (mainly due to glucose and fructose) was detected in watercress and rocket salad (8.44 and 7.98 mg GLU/g FW, respectively), while daikon and broccoli contained an almost halved content (4.47 and 4.66 mg GLU/g FW respectively) of these compounds. Considering the total sugar content, mustard showed the highest content by a significant amount (58.11 mg GLU/g FW), threefold higher than that of broccoli, rocket salad, and watercress, and twofold higher than that of daikon.

The growth condition of the microgreens can be evaluated by their chlorophyll content. The highest (statistically different) content of chlorophyll a (Chl a) was found in mustard (982.3 μg/g FW), followed by broccoli (737.8 μg/g FW), and rocket salad, daikon, and watercress, which showed similar values to one another (681.8, 623.6 and 584.8 μg/g FW, respectively). A similar trend was observed for the content of chlorophyll b (Chl b), of which mustard showed the highest level (409.2 μg/g FW), followed by watercress (233.0 μg/g FW) and broccoli (223.9 μg/g FW), while its lowest concentration was found in daikon and rocket salad (170.3 and 131.8 μg/g FW, respectively). The total chlorophyll content reflects the sum of the single chlorophyll

content, of which mustard showed the highest content, while daikon showed the lowest. The Chl a/Chl b ratio was in the range of 2.51–3.66, with the exception of rocket salad (5.22); the generally accepted ratio which is considered an index of optimal plant growth is above 2.5–3.

The total carotenoids amount was significantly lower in watercress (96.9 μg/g FW), while the other Brassicaceae microgreens showed similar values, in the range of 175–217 μg/g FW.

In the evaluation of their nutritional value, antioxidant compounds such as polyphenols, anthocyanins, AsA, and carotenoids play an important role, and are associated with the antioxidant activity. The highest total polyphenol content (TPC) was detected in broccoli (3.63 μg/g FW), followed by daikon, watercress, and rocket salad. Mustard showed the lowest amount (1.02 μg/g FW), which was statistically different from the other microgreens.

The highest level of anthocyanins was found in mustard (405.52 μg/g), followed by broccoli (172.51 μg/g FW). The lowest levels, on the other hand, were detected in daikon and watercress (57.56 and 52.28 μg/g FW, respectively). Rocket salad (42.26 μg/g FW) showed the lowest anthocyanin concentration, which was statistically different from the other species.

The largest vitamin C (total AsA) content was detected in mustard (606.87 μg/g FW). In broccoli, daikon, and watercress, the AsA content was about fourfold lower than that in mustard, but very similar to each other (124.1–137.52 μg/g FW). A rather low content was detected in rocket salad (29.67 μg/g FW). Lastly, the reduced AsA content was extremely high in mustard (366.07 μg/g FW), and about 10 times lower in rocket salad (25.86 μg/g FW) and watercress (38.55 μg/g FW).

The highest antioxidant activity was observed in daikon, broccoli and watercress, in both the DPPH and FRAP assays, while rocket salad and mustard showed significantly lower activities.

### 3.16.MINERAL ANALYSIS

The mineral element composition was analyzed for 30 varieties of microgreens, representing 10 species within 6 genera of the Brassicaceae family. Brassicaceae microgreens were assayed for concentrations of macroelements, including calcium (Ca), magnesium (Mg), phosphorous (P), sodium (Na), potassium (K), and of microelements, including copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). Determinations of mineral elements in microgreen samples were performed using an inductively coupled plasma optical emission spectrophotometer (ICP OES). Potassium was the most abundant macroelement ranging from 176 to 387 mg/100 g fresh weight (FW), followed by P (52–86 mg/100 g FW), Ca (28–66 mg/100 g FW), Mg (28–66 mg/100 g FW), and Na (19–68 mg/100 g FW). Among the microelements, Fe tended to be most abundant (0.47–0.84 mg/100 g FW), followed by Zn (0.22–0.51 mg/100 g FW), Mn (0.17–0.48 mg/100 g FW), and Cu (0.041–0.13 mg/100 g FW). Based upon the analysis of 30 varieties, the results demonstrate that microgreens are good sources of both macroelements (K and Ca) and microelements (Fe and Zn.). Consumption of microgreens could be a health-promoting strategy to meet dietary reference intake requirements for essential elements beneficial to human health.(Xiao *et al*.,2016)

# CHAPTER 4

# MATERIALS AND METHODS

Microgreens are easy to grow, quick to harvest which are packed with flavors and loaded with nutrients. They are developed in daylight and soil. They can be grown inside on a sunny window rack or in a shade house. They are grown from microgreen seeds which are accessible in the business all round the year. The harvest period of microgreens is 7 to 14 days and they do not need manures or pesticides.

## 4.1.REQUIREMENTS

### 4.1.1; MICROGREEN SEEDS

Microgreens cannot be grown from regular seeds, one should use microgreen seeds only. In this study common microgreen seeds like green mustard, radish pink, spinach, sunflower and wheat grass seeds were used.

#### 4.1.1.1; SEED COLLECTION

Microgreen seeds were bought online from Bombay Hydroponics (plate 1). Six types of seeds where bought. They were green mustard, radish pink, radish white, spinach, sunflower and wheat grass.

**Plate 1: Soaked microgreen seeds ready to sow**

### 4.1.2; POTTING MEDIUM

Cocopeat was the chief medium used in this experiment (plate 2). It was made from pith inside a coconut husk. It is a  natural antifungal and has high water holding capacity. It is an excellent medium for the growth of microgreens as it promote strong root growth and also very affordable. Along with it Vesicular arbuscular mycorrhiza (VAM) was associated with the cocopeat (plate 3).VAM is formed by the symbiotic association between phycomycetous fungi and angiosperm roots. It colonizes the root cortex forming a mycelial network thereby protecting it.

**Plate 2: Sterilized cocopeat**

**Plate 3:VAM**

### 4.1.3; TRAY

A container to grow microgreens with 4-5 inches deep (plate 4).They possess holes for draining excess water and could also be used to water the tray from underneath.

**Plate 4: Tray for microgreen cultivation**

### 4.1.4; LIGHT SOURCE

Microgreens required an essential amount of sunlight /natural light for at least 3-4 hours a day. A bright windowsill or a balcony that receives sunlight will be a good spot for the plant.

### 4.1.5; WATER

Water is a necessary element for Microgreens. The soil being used is to be kept moist at all times. A hand sprinkler works best for the plant,due to its micro-outlets thatdo not let opena huge downpour of water.

Microgreens were rinsed 2 times according to the dryness of the potting medium to water clogging which may spoil the microgreens. They were typically grown in adark environment until they germinate.Theywere transferred to an area where they can get atleast six hours of sunlight per day.

## 4.2; <u>STEPS IN CULTIVATION OF FRESH, FLAVOURFUL AND HEALTHY MICROGREENS</u>

## 1. STERILIZATION OF COCOPEAT:

Cocopeat block could be first soaked in water for few minutes followed by boiling it in a container in low flame or could be oven heated to 180 degrees F for 30 minutes. Finally it could be dried and used as the potting medium. Sterilization helps to prevent the possibility of spreading disease which are harmful to our plants. Thus complete aseptic conditions could be maintained.

## 2. FILLING THE TRAY

Since the roots of Microgreens do not reach that deep, 3-4 inches soil height should be good enough. Here two substrates were used. Five trays were set up for cocopeat only and other five for VAM associated cocopeat.Small holes were made with fingers on the tray and one to two grams of VAM also added to it.

## 3. SOW THE SEEDS

Microgreen seeds were spread on the soil surface. The spacing between the seeds does not need to be completely even, so hand sprinkling works fine.

## 4. COVER THE SEEDS:

Cover with a very thin layer of soil and gently pat the surface in order to make the seeds settle well in the container. Light is not necessary for first two days for seed germination.

## 5. DAMP THE SOIL

Sprayed enough water over the soil surface in order to make the surface completely moist, but do not flood it with water.

## 6. LIGHT CONDITIONS:

Kept the container for about two days until germination occurs. Then choose a sunny spot to place the plant, where it receives a good amount of sunlight for at least 3-4 hours in a day.

**7. SPRINKLE WATER TWICE**:

Sprinkled the water over the growing greens, twice a day. In 3-4 days time, small leaves grow over the soil with little shoots at the bottom.

**8. HARVESTING MICROGREENS**

Once the plants were 2-3 inches taller, they are ready to be harvested. Cut the microgreens with a pair of scissors or a sharp knife and cut the Microgreens, holding them vertically, from just over the roots.

**9. WASHING:**

Wash the harvested microgreens with cold running water and used it in a meal. Microgreens provide the best of nutrition when consumed fresh, right after the harvest. Dried them after washing and stored in a paper wrap in the refrigerator.

## 4.3; DETERMINATION OF MOISTURE CONTENT

Method relies on measuring the mass of water in a known mass of sample before and after the water is removed by evaporation.

$$\text{Moisture (\%)} = \frac{W1-W2}{W1} \times 100$$

Where, W1 = Weight (g) of the sample before drying

W2= Weight (g) of the sample after drying

## 4.4; DETERMINATION OF %OF SOLID WEIGHT

The basic principle of this technique is that water has a lower boiling point than the other major components within food material ,e.g., lipids, proteins, carbohydrates and minerals. Sometimes a related parameter, known as the total solids, is reported as a measure of the moisture content.

$$\text{Solid Weight (\%)} = \frac{W2}{W1} \times 100$$

Thus, % total solids= 100 - %moisture

Where, W1 = Weight (g) of the sample before drying

W2= Weight (g) of the sample after drying

## 4.5; PREPARATION OF PHOSPHATE BUFFER SOLUTION (pH) FOR EXTRACTION

Prepared 25 mL of distilled water in a suitable container and add 0.2 g of Sodium chloride to the solution then added 5 mg of potassium chloride to the solution. Afterwards added 36 mg of sodium phosphate dibasic to the solution and 6.125 mg of potassium phosphate monobasic to the solution.

## 4.6; QUALITATIVE ESTIMATION OF PHYTOCOMPOUNDS

**a. Shinoda test**

Magnesium turnings and diuted. HCl were added to the extract, dissolved in alcohol. Formation of deep red colour indicated the presence of flavonoids.

**b. Mayer's test**

Few drops of Mayer's reagent ($K_2HgI_4$) were added to the extract and the formation of creamy white precipitate indicated the existence of alkaloids.

**c. Molisch's test**

The extract in alcohol was mixed with a few drops of Molisch's reagent (alpha naphthol). Concentrated $H_2SO_4$ was added along the sides of the test tube. Formation of purple coloured ring at the junction indicated the presence of carbohydrates.

**d. Ferric Chloride test**

A few drops of 5% ferric chloride were added along the side of the test tube to a mixture of 200 µL of the extract and 2 mL of distilled water. A dark green colour marked the presence of phenolic compounds.

**e. Salkowski test**

To the alcoholic extract added equal amount of chloroform. Slightly warm and when cooled added con $H_2SO_4$ through the sides of the test tube. A reddish-brown ring form at the interface indicated presence of terpenoid. If green colouration developed below the interface, then it marked the presence of steroids.

**f. Foam test**

10ml of distilled water was added to the extract and shaken well for few minutes. Formation of frothing lasting for 60-120 seconds indicated the presence of saponins.

**g. Test for Fixed Oils and Fats(Spot test)**

A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oils and fats.

## 4.7; ANTINUTRITIONAL ANALYSIS

### 4.7.1; TOTAL PHENOLIC CONTENT (TPC)

Total natural phenolic content present in the aqueous extract was determined using Folin -Ciocalteu reagent (Singleton and Rossi, 1965).An aliquot of 0.5 mL (Con.100µg) of the extract was treated with 2.5 mL of Folin – Ciocalteu's reagent. Mixed thoroughly and after 5 minutes 2 mL of 7.5% sodium carbonate ($Na_2CO3$) solution was added. The reaction mixture was kept for incubation at 45˚C for 15 min. The blue colour developed was read at 765 nm.

Gallic acid was used as the reference standard and the TPC was estimated from the calibration graph of Gallic acid. The total phenolic content (TPC) in the extracts were calculated using the equation $T = C \times (V/M)$ where, T is the total phenolic content (mg/g) of the extracts, C is the concentration of Gallic acid (mg/mL) obtained from the calibration graph, V is the volume of the extract taken (mL) and M is the weight of the extract (g). The results were expressed as milligram Gallic acid equivalents (mg GAE)/g of sample dry weight (Singleton *et al.*,1965).

### 4.7.2; TOTAL FLAVONOID CONTENT

Total flavonoid content in the extract was evaluated by AluminiumChloride colorimetric method according to Woisky and Salatino (1998). To 0.1 mL of extract, 0.3 mL of methanol, 0.02mL of 10% $AlCl_3$, 0.02 mL of 1 M potassium acetate ($CH_3COOK$) and 0.56 mL of distilled water were added. The mixture was kept for incubation at room temperature for 30 minutes. The absorbance was measured at 415 nm. Quercetin was kept as the standard. TFC was calculated from the calibration graph plotted for Quercetin using the formula T = C x (V/M) where T is the total flavonoid content (mg/g) of the extracts, C is the concentration of Quercetin (mg/mL) obtained from the calibration graph, V is the volume of the extract taken (mL) and M is the weight of the extract (g). TFC is expressed as milligram Quercetin equivalent (mg QE)/g sample dry weight.(Woisky *et al.*,1998)

## 4.8; NUTRITIONAL ANALYSIS

### 4.8.1; ESTIMATION OF PROTEIN BY LOWRY'S METHOD

**RRQUIREMENTS :**

•Reagent A : 2% Sodium carbonate dissolved in 0.1 N sodium hydroxide

•Reagent B : 0.5% Copper sulphate.

•Reagent C: Alkaline copper solution: about 50 mL of reagent A was mixed with 1 mL of reagent B prior to use.

•Folin-Ciocalteau reagent. Phenol reagent was mixed with equal volume of water before use.

•Stock solution (Protein solution): 50 mg of Bovine Serum Albumin (BSA) was dissolved and made upto 50 mL with distilled water in a standard flask.

•Working standard: 10 mL of the stock solution was diluted to 50 mL with distilled water in a standard flask.

**PROCEDURE :**

To a series of test tubes marked S1 to S5, 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL and 1.0 mL of working standard was pipetted out. 1 mL test solution (supernatant of the treated sample) was taken into test tubes marked T1, T2. Make up the volume to 1mL in required test tubes with distilled water. Distilled water was taken as the blank. 5 mL of Reagent C was added to all test tubes and mixed well. Incubated for 10 minutes at room temperature and then added 0.5mL of Folin-Ciocalteau reagent to all test tubes. Mixed well and incubated at room temperature in dark for 30 minutes. The blue colourdevelopedwas read at 660nm.

## 4.8.2; ESTIMATION OF TOTAL CARBOHYDRATE

**REAGENTS :**

- 5% phenol in distilled water.
- 96%sulphuricacid in distilled water.

**PROCEDURE :**

0.2,0.4,0.6,0.8 and1ml of working standard (with 0.1mg/ml conc.) of glucose was taken inboiling tubes and the final volumes of each tube was made up to 1ml by adding distilled water.1ml of 5% Phenol and 5ml of 96% sulphuric acid was added one by one in each tubes and shookwell so that the Phenol and Sulphuric acid get mixed thoroughly with working standard.After 10minutes all the tubes were placed in water bath at 25-30°C for 15 minutes. Blank was set with1ml of distilled water and O.D. of each tube was taken at 490nm with the help ofspectrophotometer.(Masuko. *etal.*,2005)

## 4.8.3; ESTIMATION OF LIPID

**REAGENTS :**

- Chloroform
- Methanol
- 0.73% sodium chloride in distilled water.

**PROCEDURE :**

Total lipid extraction performed by conventional extraction methods, relying on the chloroformmethanol solvent system is a faster single-step procedure was developed for extraction of total lipids from sample. In the single-step procedure, 8 ml of a 2:1 chloroform-methanol (v/v) mixture was added to dried powdered sample(Wi) contained in a centrifuge tube. The biomass was manually suspended by vigorously shaken the tube for a few seconds and 2 ml of a 0.73% NaCl water solution was added. Phase separation was facilitated by 2 min of centrifugation at 350 g. and the lower phase was recovered for analysis. The chloroform extract was separated in preweighed glass vials and waited for its complete drying (Wf). Chloroform was evaporated and the yield (%) of total lipid contents was measured by the formula (Bligh and Dyer *etal*.,1959).

$$\text{Total lipid (\%)} = \frac{Wf}{Wi} \times 100$$

**4.9 ; TOTAL ANTIOXIDANT CAPACITY (TAC)**

The total antioxidant capacity of different extracts was evaluated by Phosphomolybdic Acid method (Prieto *et al*., 1999). To 0.1 mL of the extract (Con.100µg) 1mL reagent mixture containing 0.6M Sulphuric Acid, 28 mM Sodium Phosphate and 4mM Ammonium Molybdate were added. The tubes were covered and kept for incubation in a water bath at 95˚C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm against blank. Gallic acid was used as the standard and the total antioxidant capacity was calculated using from the equation $T = C \times (V/M)$ where, T is the total antioxidant content (mg/g) of the extracts, C is the concentration of Gallic acid (mg/mL) obtained from the calibration graph, V is the volume of the extract taken (mL) and M is the weight of the extract (g). TAC is expressed as milligram Gallic acid equivalents (mg GAE)/g of extract ( Prieto *et al*.,1999).

**4.10; DPPH (1–1-diphenyl–2–picryl hydrazine) (FREE RADICAL SCAVANGING ASSAY)**

Quantitative measurement of radical scavenging property of extract was carried out according to the method of Mensor*et al*., (2001). A methanolic solution of 1 mL of DPPH (0.1 mM) was added to 1 mL of different concentration (50-1000 µg/mL) of the extract and allowed to react at room temperature for 30 min in the dark. Absorbance was measured at 517 nm. Methanol served as the blank, DPPH in methanol without the extract served as the control and ascorbic acid was taken as the reference standard. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capacity to scavenge the DPPH radical was calculated using the following equation :-

% radical scavenging activity = (Abs.control- Abs. sample) x 100

Abs. control

A graph was plotted with percentage of inhibition against concentration. The IC50 values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation of the graph. Smaller the IC50 higher will be the antioxidant activity of the extract (Mensor *et al*.,2001).

## 4.11; MINERAL ANALYSIS – DETERMINATION OF POTTASSIUM

### 4.11.1.SAMPLE PROCESSING

The sample, if not already a solution, must be converted to a media which is suitable fordirect introduction into the flame photometer i.e. the sample should be aqueous with no solid matter present.

This is achieved by:

Extraction of salts from solid samples using deionized water. Extraction was made more successfully using a blender macerator or shaking machine. If the sample was organic then the organic material should be removed by ashing. The remaining oxides were then dissolved using strong acids.Filtration/centrifugation was done to remove solid debris.When aqueous, the sample could then be diluted to a known, accurately measured volume usingdeionized water. If it is a concentrated sample then the dilution ratio should be increased. Ifthe sample concentration is low then a small volume of diluent and initial extractant should be used.

### 4.11.2.PREPARING FOR ANALYSIS

**Initial precautions**

It is of the utmost importance to become familiar with the flame photometer and ancillary equipment prior to analysis. If familiarity is not achieved, inaccuracy of results or even a hazard to safety could result. Therefore, always read the instrument's instruction manual. There are several practical points which should be adhered to achieve the required accuracy in your analysis. Avoid handling samples with fingers. This leads to serious contamination, e.g. if a finger is immersed in 20 ml of deionised water the resulting Na concentration will exceed that of a 10ppm standard. Standards should be stored in sealed vessels and in high concentrations, i.e. store the standards as a stock 1000 ppm solution and prepare dilutions when required. The long term storage of low concentration standards is not recommended due to degradation of the ionic species.All analyses involve the use of a diluent which is almost always deionised water. This should be of the highest quality for accurate flame analysis. Sodium, potassium and calcium are present in high concentrations in tap water and thus efficient deionization is essential if any of the common flame analyses are to be determined. Standards and samples should not be exposed to the atmosphere for long periods due to contamination from airborne particles and the evaporation of the solvent leading to elevated concentrations.

**4.11.3; MAKING MEASUREMENT**

Prepared standard and sample solutions as mentioned in the result table. Power up the flamephotometer in accordance with the instruments instruction manual. Set blank with the diluents used for sample and standard preparation. This is usually deionized water . Aspirate the prepared standards in increasing concentrations and record their stable display readings. Aspirate the unknown solution and record the stable display reading. Operate the instrument shutdown procedure.

**4.12 ; RECIPIE**

**4.12.1; MICROGREEN SALAD**

**Ingredients Quantities**

Microgreens - 100g

Orange peeled - ½ cup

Apple - ½ cup

Shredded carrot - 1 cup

Cucumber - 1 cup

Pomegranate - ½ cup

**For the Dressing** :

Cold-pressed olive oil - 1 Tbsp

Lemon juice - 1 Tbsp

Honey - 2 Tbsp

Salt and pepper - To taste

**Procedure:**

- If your microgreens have some soil on them, give them a lightwash and air dry them in a strainer for a few moments.(Theyare very fragile so need to be handled with care).

- Placed them in a bowl and added the remaining salad ingredients.

- Stirred up your dressing in a little jar and pourd on top of the salad.

# CHAPTER 5

## RESULTS AND DISCUSSIONS

Microgreens, packed with nutrients were grown well in different colours and were ready to harvest in about 2 weeks. The following table 1 portrays the

variation in different microgreens that were chosen to grow with respect to the given growth conditions.

**Plate 5 : Experimental setup with only cocopeat in large tray and cocopeat+ VAM in small trays.**

**Plate 6 : Wheatgrass seeds sown in tray**

**Plate7 : Germinated microgreen tray**

| Sl. No | Variety | Seed Soaking Time | Germination Time | Harvest | Potting Medium | Length Of Shoot | No.of Leaves |
|---|---|---|---|---|---|---|---|
| 1. | Green mustard | 2 hours | 2-3 days | 5-14 days | coco peat | 7 cm | 2 |
| | | | | | cocopeat+ vam | 8 cm | 2 |
| 2 | Radish pink | 2 hours | 2-3 days | 6-10 days | cocopeat | 11 cm | 2 |
| | | | | | cocopeat+ vam | 12 cm | 2 |
| 3 | Spinach | 4-5 hours | 4-5 days | 7-10 days | cocopeat | 6 cm | 2 |
| | | | | | cocopeat+ vam | 7 cm | 2 |
| 4 | Wheat grass | overnight or 12 hours | 2-3 days | 10-14 days | cocopeat | 10 cm | 1 |
| | | | | | cocopeat+ vam | 11 cm | 1 |
| 5 | Sunflower | 24 hours | 2-4 days | 8-14 days | cocopeat | 9 cm | 2 |
| | | | | | cocopeat+ vam | 10 cm | 2 |

**Table 1 : Physical observations of grown microgreens**

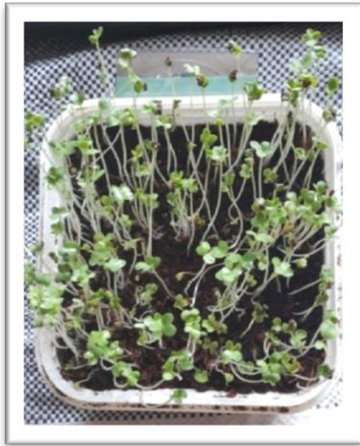
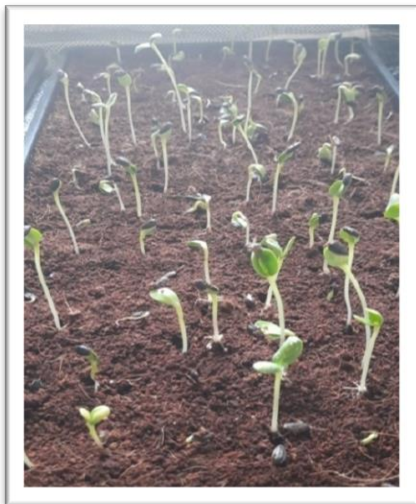


**Plate 8 :**

**Growing microgreens**

**Plate 9 : Germinated sunflower**



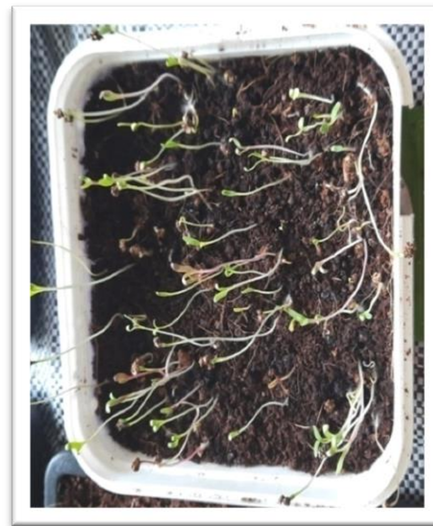


**Plate 10 : Growing sunflower microgreens.**

**Plate 11: Germinated spinach**

**microgreen**

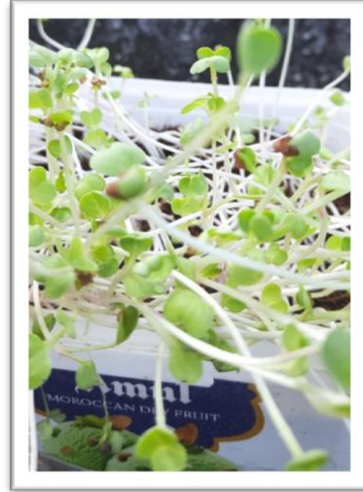


**Plate 12 : Growing wheatgrass microgreens**

**Plate 13 : Radish microgreens**

## 5.1; DETERMINATION OF MOISTURE

Initial weight (W1) = 5g

Final dry weight (W2) = 0.5 g

$$\text{Moisture \%} = \frac{5-0.5}{5} \times 100$$

$$= 90\%$$

Thus 90% of water content is present in the microgreen sample.

## 5.2; DETERMINATION OF % OF SOLID WEIGHT

$$\text{Solid weight \%} = \frac{W2}{W1} \times 100$$

$$= \frac{0.5}{5} \times 100$$

$$= 10\ \%$$

Thus 10% solid weight is contained in the microgreen sample.

## 5.3; EXTRACTION

Phosphate buffer saline(pH )was used for the extraction of the harvested microgreens. Followed by straining them through a cheese cloth and was stored in eppendorf tubes . They were labeled accordingly and stored in the deep freezer.

**Plate 14: Extracted microgreen samples**

## 5.4.; PRELIMINARY PHYTOCHEMICAL SCREENING

Various phytochemical tests were conducted to investigate the presence of flavoniods, phenolic compounds, tannins, carbohydrates,alkaloids and 19 which are the most important bioactive components that are exploited for drug discovery.

| SL.NO: | PHYTOCHEMICAL TEST | RESULT |
|---|---|---|
| 1 | SALKOWSKI TEST | SFC>SFV>RPC>RPV>GMC>GMV>SPC >SPV>WGC>WGV |
| 2 | MAYER'S TEST | RPC>RPV>GMC>GMV>SFC>SFV |
| 3 | MOLISCH'S TEST | ALL POSITIVE |
| 4 | SHINODA TEST | POSITIVE FOR GMC,GMV,RPC,RPV only. |
| 5 | FERRIC CHLORIDE TEST | ALL POSITIVE |

| 6 | FOAM TEST | POSITIVE |
|---|---|---|
| 7 | SPOT TEST | POSITIVE |

**Table 2 : Result of phytochemical test of samples**

(Where GMC-green mustard coco, GMV- green mustard VAM, RPC- Radish pink coco, RPV-Radish pink VAM, SPC-Spinach coco, SPV-Spinach VAM, SFC-Sunflower coco, SFV-Sunflower VAM, Wgc- Wheat grass coco, WGV-Wheat grass VAM)

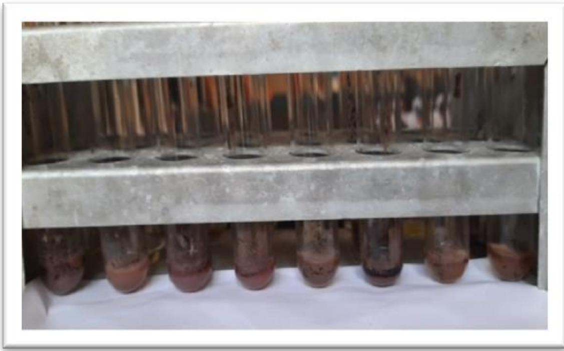


**Plate 15: Observation of Molisch test in microgreen samples**.

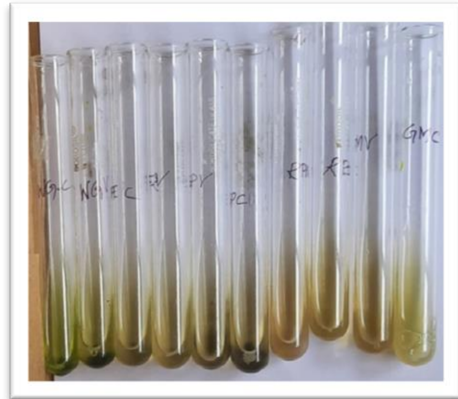


**Plate 16: Samples tested for ferric chloride test**

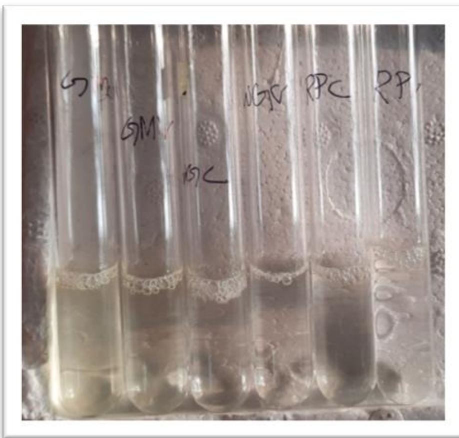



**Plate 17 :Positive result of foam test**

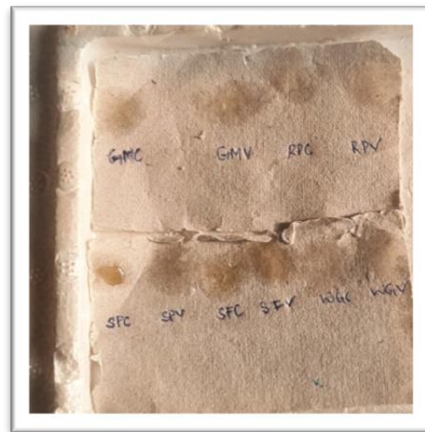



**Plate 18: Spot test**

**Plate 19: Result in spot test**

Thus, all samples other than spinach and wheatgrass microgreens exhibited a positive salkowski test. Test for alkaloids were positive only for radish pink, green mustard and sunflower microgreens. Also, all samples had ample amount of carbohydrates and flavanoids.

## 5.5. ANTINUTRITIONAL ANALYSIS

Preliminary phytochemical screening showed the presence of phenols and flavonoids in every microgreen samples.Thus, the present investigation was undertaken to estimate total phenols and flavonoids in the samples. *In vitro* antioxidant activity was also evaluated in the extract using DPPH method.

### 5.5.1; QUANTITATIVE ESTIMATION OF PHENOL

| Concentration(mg/ml) of Standard Gallic acid | Mean Absorbance @765nm |
|---|---|
| 0.01 | 0.747 |
| 0.025 | 0.89 |
| 0.05 | 1.16 |
| 0.075 | 1.3 |

| 0.1 | 1.62 |
|---|---|

**Table 3: Absorbance of gallic acid at various concentrations**

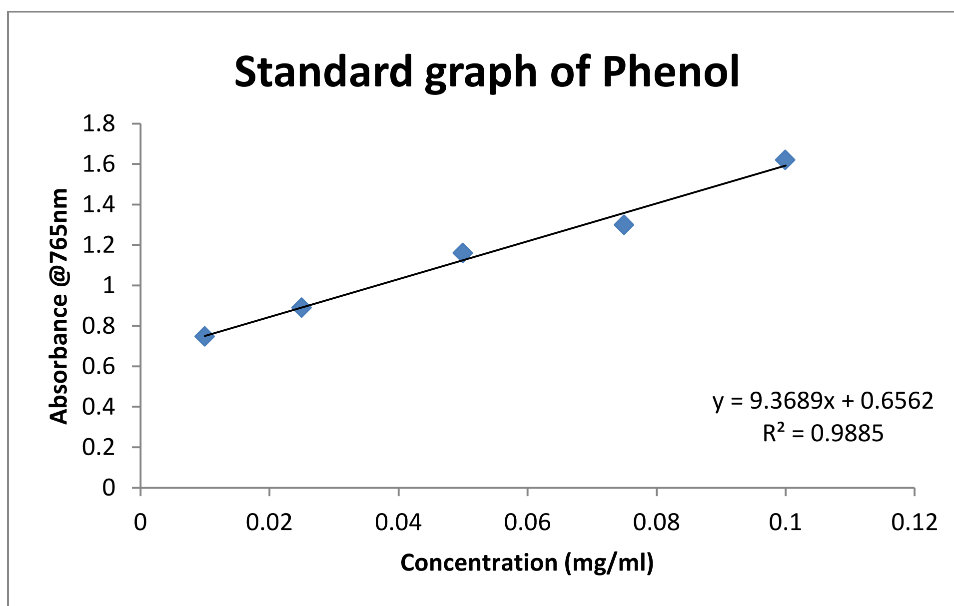



**Fig 1: Standard graph of phenol**

| SAMPLES | Mean Absorbance @ 765 nm | Concentration(mg/ml) |
|---|---|---|
| GREEN MUSTARD COCO | 1.470 | 0.087 |
| GREEN MUSTARD VAM | 1.129 | 0.050 |
| RADISH PINK COCO | 1.512 | 0.091 |
| RADISH PINK VAM | 1.599 | 0.100 |
| SPINACH COCO | 0.609 | 0.050 |
| SPINACH VAM | 0.579 | 0.082 |
| SUNFLOWER COCO | 1.504 | 0.090 |

| SUNFLOWER VAM | 1.025 | 0.039 |
|---|---|---|
| WHEATGRASS COCO | 1.243 | 0.062 |
| WHEATGRASS VAM | 0.977 | 0.034 |

**Table 4: Phenolic concentrations of samples**

**Plate 20 :Samples tested for Phenol estimation**.

As a result, phenol concentration for the microgreens were in the following order :

RPV>RPC>SFC>GMC>WGC>GMV>SFV>WGV>SPC>SPV.

## 5.5.2; ESTIMATION OF TOTAL FLAVANOID BY ALUMINIUM CHLORIDE CALORIMETRIC METHOD

A colorimetric assay using aluminum chloride was reported by Woisky and Salatino (1998) as a method that able to detect flavonoid in flavone and flavonol group, such as quercetin. Aluminum chloride in the assay will react with quercetin and create a stable, color-signature complex .The reagents used in this test are 10% $AlCl_3$ and potassium acetate. Quercetin was the standard.

| Concentration(mg/ml)of Standard (Quercetin) | Absorbance@415nm |
|---|---|
| 0.01 | 0.271 |
| 0.025 | 0.31 |
| 0.05 | 0.353 |
| 0.075 | 0.455 |
| 0.1 | 0.544 |

**Table 5 : Absorbance of quercetin at various standards**

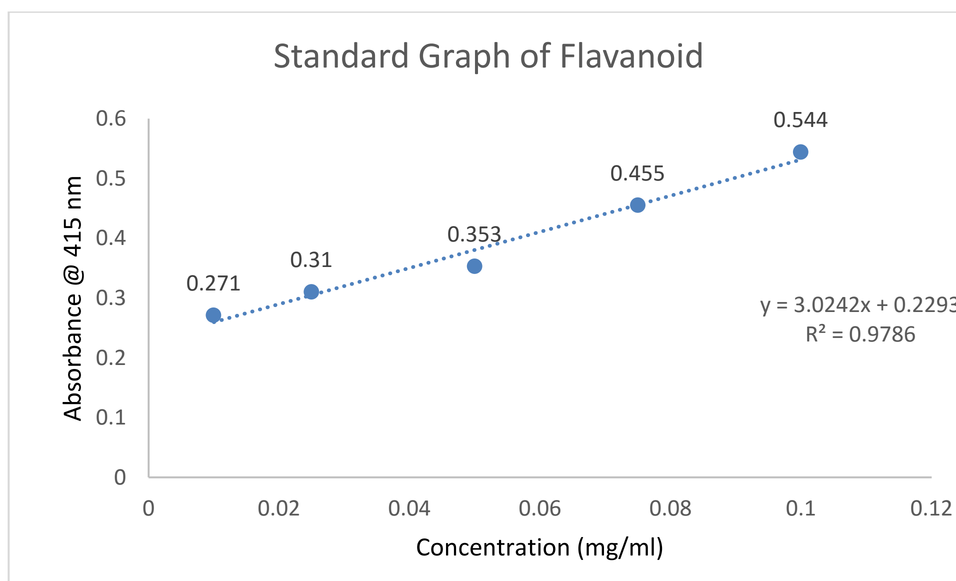



**Fig 2: Standard graph of flavanoid**

| SAMPLES | Mean Absorbance @ 415 nm | Concentration(mg/ml) | TFC ( mg quercitin equivalent/gof extract) |
|---|---|---|---|
| GREEN MUSTARD COCO | 0.316 | 0.028 | 2.8 |
| GREEN MUSTARD VAM | 0.264 | 0.011 | 1.1 |
| RADISH PINK COCO | 0.302 | 0.024 | 2.4 |
| RADISH PINK VAM | 0.416 | 0.061 | 6.1 |
| SPINACH COCO | 0.311 | 0.027 | 2.7 |
| SPINACH VAM | 0.260 | 0.010 | 1 |
| SUNFLOWER COCO | 0.377 | 0.048 | 4.8 |
| SUNFLOWER VAM | 0.253 | 0.007 | 0.7 |
| WHEATGRASS COCO | 0.4 | 0.056 | 5.6 |
| WHEATGRASS VAM<br>**T** | 0.233 | 0.0012 | 0.12 |

**Table 6 : Flavanoid concentrations of samples**

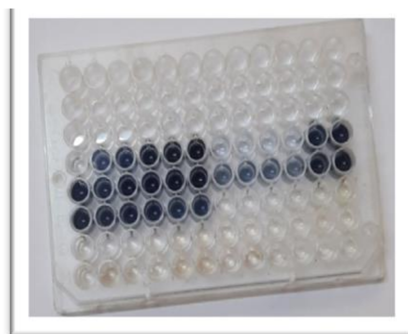


**Plate 21: Microtiter well tested for flavanoid**

As a result, Radish pink VAM microgreen has the highest flavanoid content. Their increasing order is as given below:

RPV>SFC>GMC>SPC>RPC>GMV>SPV>SFV>WGV>WGC.

## 5.6; NUTRITIONAL ANALYSIS

### 5.6.1; CARBOHYDRATE ESTIMATION BY PHENOL SULPHURIC ACID METHOD

In this study , all the five samples possess ample amount of carbohydrate content as per the spectrophotometer readings as well the golden yellow colour it showed after the test.

| Concentration(mg/ml) of standard(Glucose) | Absorbance @490nm |
|---|---|
| 0.02 | 0.132 |
| 0.04 | 0.146 |
| 0.06 | 0.152 |
| 0.08 | 0.159 |
| 0.1 | 0.165 |

**Table 7 : Absorbance of glucose at various concentrations.**

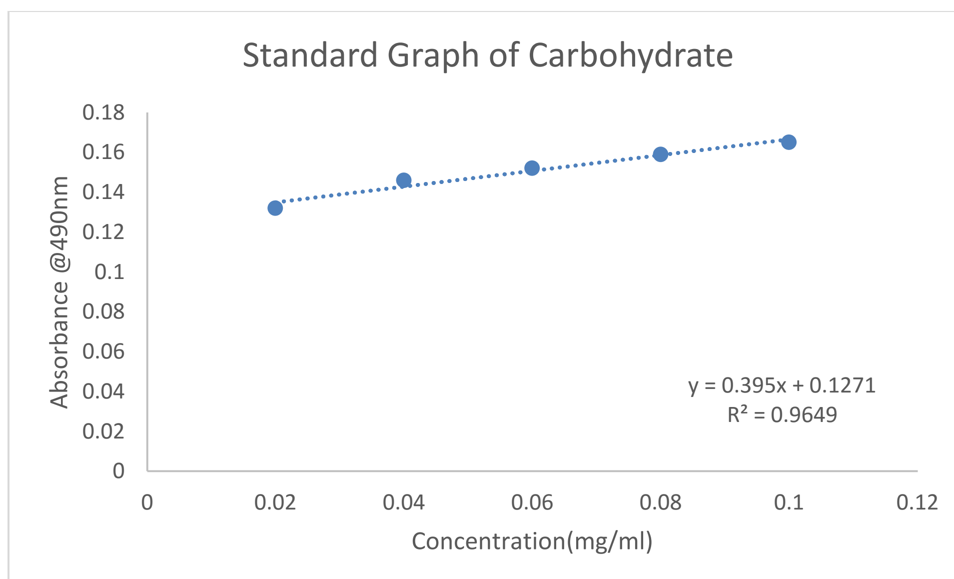



**Fig 3: Standard graph of carbohydrate**

| SAMPLES | Mean Absorbance @ 490 nm | Concentration(mg/ml) |
|---|---|---|
| GREEN MUSTARD COCO | 0.382 | 0.645 |
| GREEN MUSTARD VAM | 0.418 | 0.737 |
| RADISH PINK COCO | 0.551 | 1.073 |
| RADISH PINK VAM | 0.361 | 0.592 |
| SPINACH COCO | 0.496 | 0.934 |
| SPINACH VAM | 0.263 | 0.344 |
| SUNFLOWER COCO | 0.772 | 1.633 |
| SUNFLOWER VAM | 0.591 | 1.175 |
| WHEATGRASS COCO | 0.366 | 0.605 |
| WHEATGRASS VAM | 0.330 | 0.514 |

**Table 8: Absorbance and concentration of the samples.**

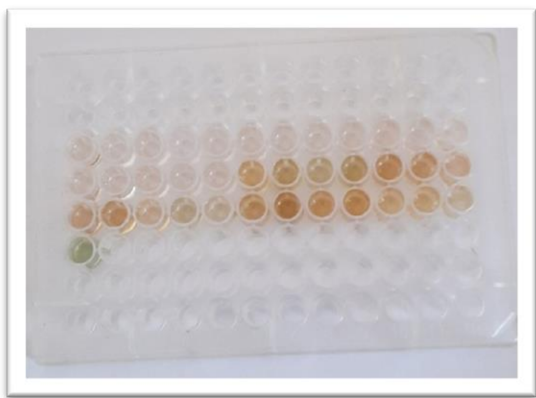


**Plate 22 : Microtiter well with samples showing presence of carbohydrate**

**Plate 23 : Quantitative estimation by spectrophotometer.**

Therefore sunflower coco has the highest carbohydrate content while Spinach VAM has the lowest. Their increasing order is as follows :

SFC>SFV>RPC>SPC>GMV>GMC>WGC>RPV>WGV>SPV.

## <u>5.6.2; ESTIMATION OF PROTEIN BY LOWRY'S METHOD</u>

All the samples were subjected to protein estimation and the OD values were obtained using spectrophotometer. The values were marked against the standard graph and concentration was calculated from unknown samples.

| Concentration(mg/ml) of Standard(BSA) | Absorbance @660nm |
|---|---|
| 0.02 | 0.07 |
| 0.04 | 0.072 |
| 0.06 | 0.075 |
| 0.08 | 0.081 |
| 0.1 | 0.083 |

**Table 9 : Absorbance of BSA in different concentrations**

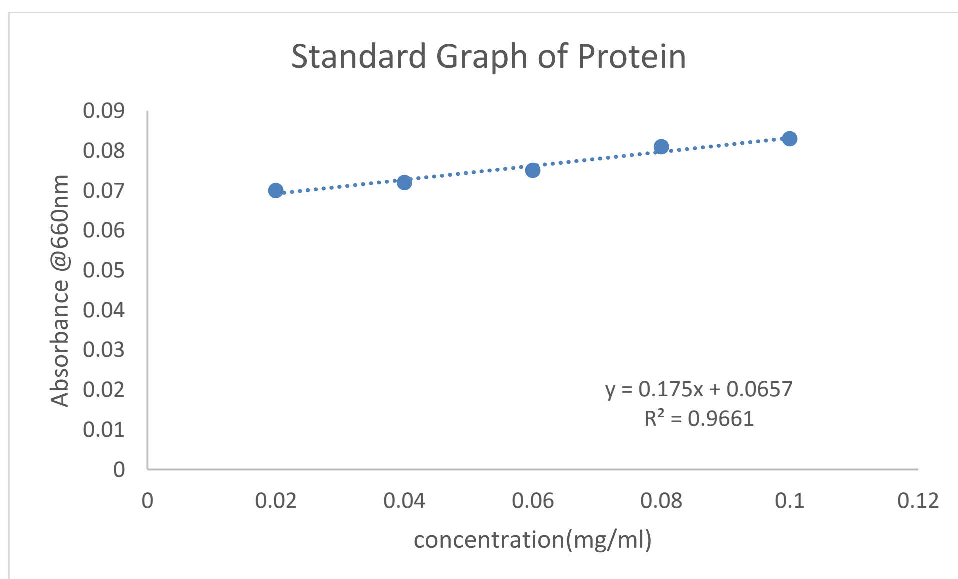



**Fig : 4 : Standard graph of Protein**

| SAMPLES | Mean Absorbance @ 660 nm | Concentration(mg/ml) |
| --- | --- | --- |
| GREEN MUSTARD COCO | 0.529 | 2.651 |
| GREEN MUSTARD VAM | 0.379 | 1.794 |
| RADISH PINK COCO | 0.565 | 2.857 |
| RADISH PINK VAM | 0.530 | 2.657 |
| SPINACH COCO | 0.252 | 1.068 |
| SPINACH VAM | 0.177 | 0.64 |
| SUNFLOWER COCO | 0.930 | 4.942 |
| SUNFLOWER VAM | 0.540 | 2.714 |
| WHEATGRASS COCO | 0.522 | 2.611 |
| WHEATGRASS VAM | 0.596 | 3.034 |

**Table 10 : Absorbance and concentartion of samples**

From this study it is concluded that sunflower microgreens have the highest protein content when compared to others. By substituting these to other non nutritive food in the diet of individuals,the nutritional quality of food can be improved and this can prevent malnutrition and protein deficiency problems. When validated with the clinical studies, the work can be implemented in diet habits of the individuals.Their increasing order is :

SFC>WGV>RPC>SFV>RPV>GMC>WGC>GMV>SPC>SPV.

**Plate 24: Samples tested for protein estimation by Lowry.**

## 5.6.3; ESTIMATION OF LIPID

$$\text{Total lipid } (\%) = \frac{W_f}{W_i} \times 100$$

| SAMPLES | % of Total lipid |
|---|---|
| GREEN MUSTARD COCO | 0.89 |
| GREEN MUSTARD VAM | 0.89 |
| RADISH PINK COCO | 0.85 |
| RADISH PINK VAM | 0.85 |
| SPINACH COCO | 0.88 |
| SPINACH VAM | 0.88 |
| SUNFLOWER COCO | 0.98 |

| SUNFLOWER VAM | 0.95 |
|---|---|
| WHEATGRASS COCO | 0.92 |
| WHEATGRASS VAM | 0.91 |

**Table 11 : Percentage of total lipid of samples**

As a result, sunflower coco microgreen has the highest lipiid content.Their increasing order is as follows :

SFC>SFV>WGC>WGV>GMC=GMV>SPC=SPV>RPC=RPV.

## 5.7; TEST FOR TOTAL ANTIOXIDANT CAPACITY

| Concentration(mg/ml) of std Ascorbic acid | Absorbance @695 nm |
|---|---|
| 10 | 0.881 |
| 25 | 1.041 |
| 50 | 1.272 |
| 75 | 1.432 |
| 100 | 1.644 |

**Table 12:Absorbance of standard at various concentration**

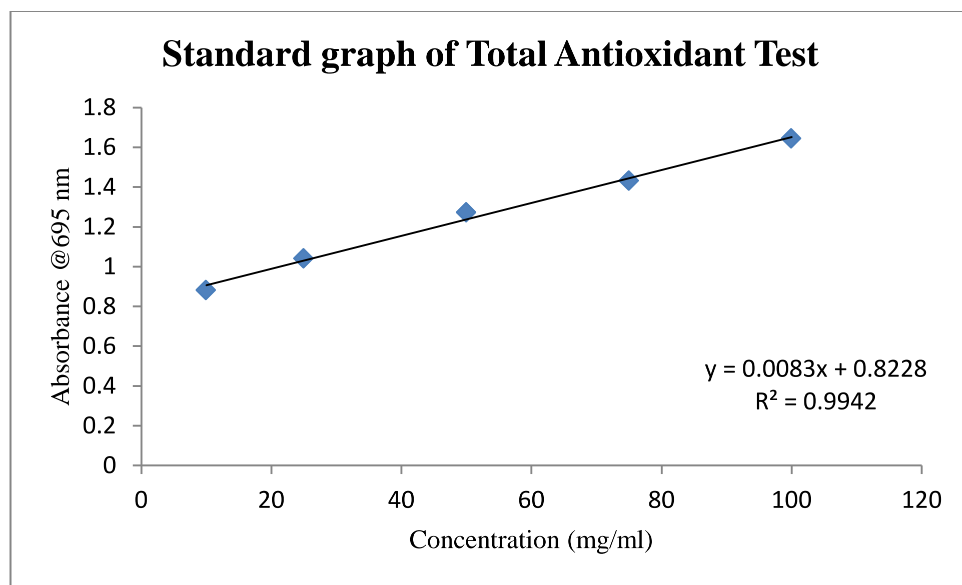



**Fig 5 : Standard graph of TAC**

| SAMPLES | Mean Absorbance @ 695 nm | Concentration(mg/ml) | TAC (mg GAE/g of extract) |
|---|---|---|---|
| GREEN MUSTARD COCO | 2.512 | 211.25 | 0.21 |
| GREEN MUSTARD VAM | 1.902 | 135 | 0.14 |
| SPINACH COCO | 0.928 | 13.25 | 0.013 |
| SPINACH VAM | 1.067 | 30.625 | 0.030 |
| SUNFLOWER COCO | 2.207 | 173.12 | 0.173 |
| SUNFLOWER VAM | 1.927 | 138.12 | 0.138 |
| WHEATGRASS COCO | 1.379 | 69.625 | 0.069 |
| WHEATGRASS VAM | 2.277 | 181.87 | 0.182 |

**Table 13: TAC of different microgreen samples**

Out of the eight samples (green mustard coco, green mustard VAM, spinach coco and VAM, sunflower coco and VAM, Wheat grass coco and Wheat grass VAM ), green mustard coco (GMC) possess the highest value 2.5. Hence,different concentration of GMC samples were taken for DPPH test.

**Plate 25 : Test for TAC**

**5.8;DPPH (1–1-diphenyl–2–picryl hydrazine) Free Radical Scavenging Assay**

| Concentration(µg/ml)of Standard (Ascorbic acid) | Mean Absorbance @517nm | % inhibition |
|---|---|---|
| 6.25 | 0.181 | 40.06 |
| 12.5 | 0.138 | 54.3 |
| 25 | 0.095 | 68.54 |
| 50 | 0.056 | 81.46 |

| 100 | 0.015 | 94.1 |
|---|---|---|

**Table 14 : Absorbance of ascorbic acid standard**

Thus, IC 50 value of ascorbic acid =10.095 ug/ml

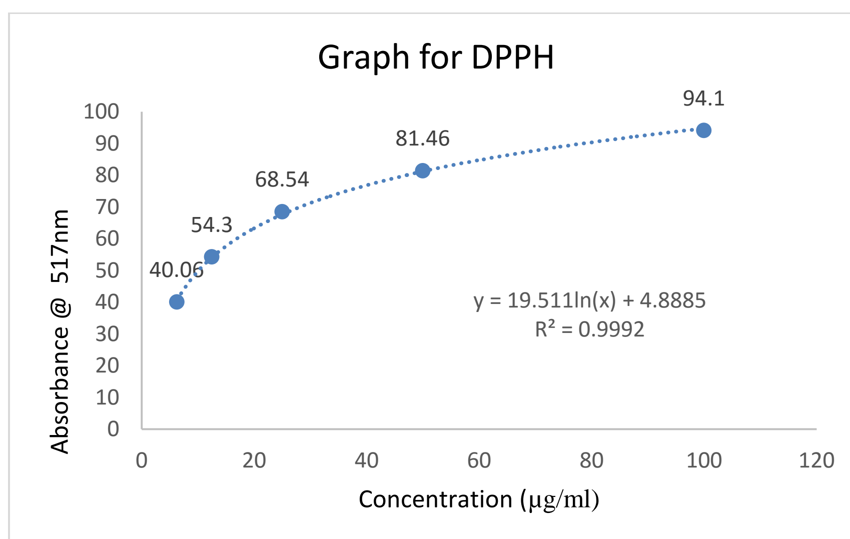



**Fig 6 : Graph for DPPH**

| SAMPLES(Green mustard coco) | Mean Absorbance @ **517 nm** | % Inhibition |
|---|---|---|
| 62.5 | 0.26 | 13.91 |
| 125 | 0.21 | 30.46 |
| 250 | 0.156 | 48.34 |
| 500 | 0.104 | 65.56 |
| 1000 | 0.051 | 83.11 |

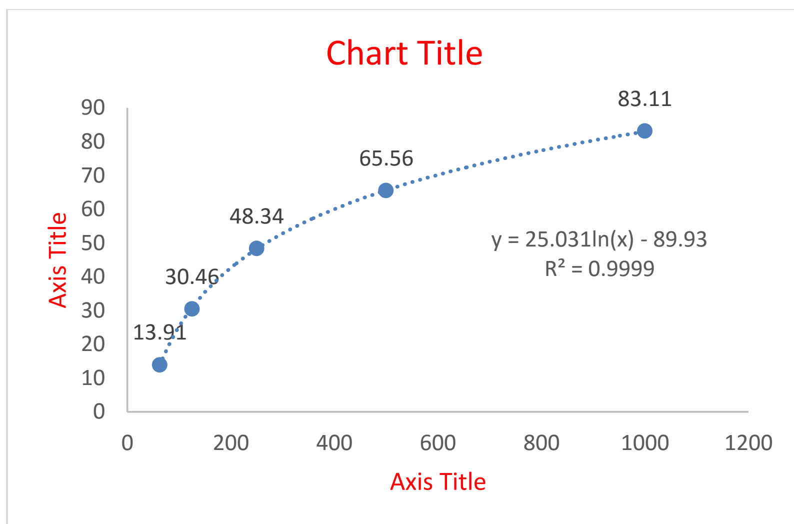



**Table 15 : Absorbance and % inhibition of various concentrations of Green mustard Coco.**

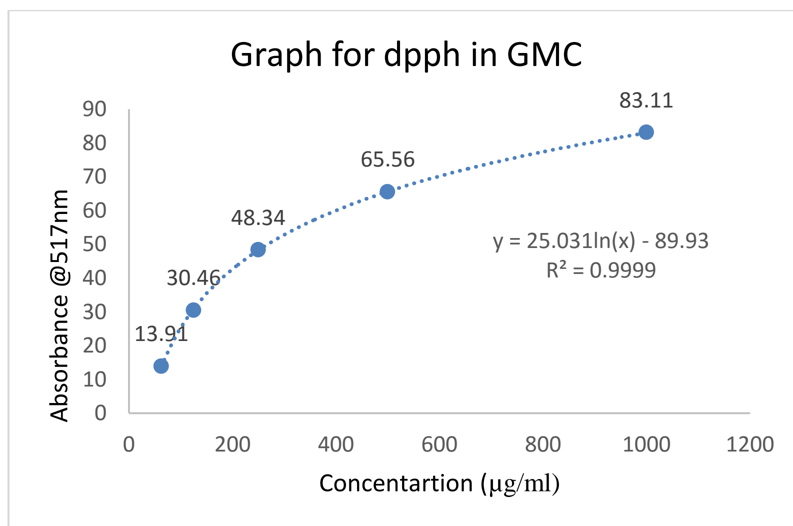



**Fig 7 : Graph for DPPH in GMC**

Therefore, IC50 value of Green mustard coco was 267.79 µg/ml.

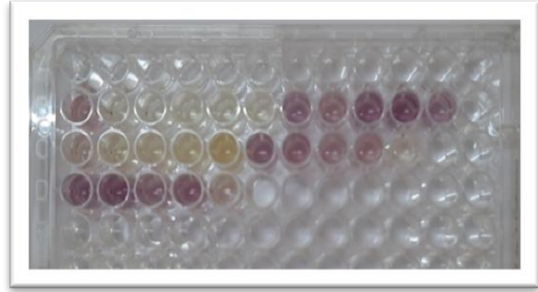


**Plate 26: Samples tested for DPPH**

**Plate 27: Microtiter well with samples**

## 5.9; MINERAL ANALYSIS – DETERMINATION OF POTTASSIUM

The sample sunflower microgreen grown in cocopeat medium ( sunflowercoco) was tested for mineral analysis. The sample was tested for potassium element which posess 93.23 ppm/10 ml test sample.

| **Sample** | **Element** | **Special preparation** | **Diluents** | **Dilution factor** | **Standards** | **Result (ppm/10ml of test sample)** |
|---|---|---|---|---|---|---|
| Sunflower Coco | Potassium | None (No ash preparation done) | Distilled water | 001 | 100 ppm, 50 ppm KCl | 93.23 |

## 5.10 ;RECIPIE : MICROGREEN SALAD

**Plate 28: Tasty, healthy and vibrant microgreen salad.**

# DISCUSSION

Microgreens ought to be gathered at the principle genuine leaf phase of plant development when seedlings are around 2 inches tall. Collecting is generally the most tedious segment of microgreen production. Microgreens are exceptionally transitory, so they ought to be taken care of with most extreme consideration following reaping. Collected microgreens are best put away in plastic compartments that shield greens from being harmed. Microgreens are entirely transient. Whenever refrigerated and avoided direct light, microgreens commonly last for 5-7 days after harvest. It is hence imperative to design production appropriately.

Microgreens are an arising class of claim to fame crops that have increased expanding consideration in the most recent decade for both their dietary and organoleptic attributes. The sorts of yields that are chosen for production and deal as microgreens have an incentive regarding color (like red or purple), texture, or flavors. Indeed, microgreens are regularly promoted as strength blends, for example, "sweet," "mellow," "brilliant," or "hot." Developing microgreens can give networks expanded food security and empower independence in homes. Microgreens are brilliant yields for urban farming in little spaces, as they can be filled in practically any climate with negligible supplies. Urban farming of microgreens gives monetary occasion to home producers that can create benefit from a little zone in their homes with negligible forthright expenses. Microgreens can be created rapidly and are right now encountering developing interest from customers that now like never before are looking for nearby, supportable hotspots for food.

The growing demand for “superfoods” like microgreens can be a good opportunity for the Indian food industry. By creating special categories for greens, more people become aware of microgreens. Microgreens have a high price market and reliable customer segment (culinary and fancy restaurants) and, its high price market is a result of expensive production costs and challenges to keep them contamination-free during all stages, starting from media preparation to proper packaging. Contamination is no more a challenge because of modern technology, however that also amounts to its expense. On the other hand, microgreens low yield, rapid senescence, and very short shelf-life curbs the expansion of their commercial production (Chandra *et al*., 2012;Kou *et al*., 2013). The COVID pandemic is actively impacting supply chains across industries. Restaurants have experienced closures or substantial drop-offs in demand, and lots of farms have adapted their business to undertake to remain afloat. Food safety,

accessibility, and quality is usually the topic of public policy and political discussion due to the role it plays in public health.. Most microgreen production is currently domestic and the 2020 pandemic is expected to decelerate demand growth for organic crops due to a decline in discretionary spending (Grooms, 2020)

The commercial production of microgreens is usually done under a controlled environment, within seedlings or high-rise corridors provided with simple or advanced technology, depending on the size of the farm and mild or favourable weather conditions, using groundless growing systems that can be caused by three types: -Growing microgreens in "containers," constituted by plastic trays having different sizes, with height variable from 3 to 5 cm. The second way is to grow microgreens in "channels" or on benches (made of plastic, aluminium, galvanized iron, wood) of various sizes, by placing growing media right inside the stations or on channels. A third growing system, quite simple but less common at commercial level, is the "floating system( Renna *et al*., 2017).

However, the growing substrates had significant effect on fresh weight of microgreens. All of the microgreen trays had good performance on growing media used in this study i.e, coco peat and VAM and no disease infection were observed during growing days. According to Bewley (1997), growing substrates with good aeration (20-30%) and optimal water holding capacity (50-70%) promoted higher seed germination. In this study, the tested growing substrates had good quality and were suitable for germination.

# CHAPTER 6

## SUMMARY AND CONCLUSION

In this study, each sample displayed high nutritional, mineral and phytochemical value. Microgreens are attributed to their low caloric composition and rich micronutrient and antioxidant composition.The present study investigated the bioactive composition and proximate analysis of five microgreens belonging to Brassicaseae, Amaranthaceae, Asteraceae, Poaceae etc. The results provide basic information and highlight the benefits of utilizing genetic biodiversity to obtain microgreens with the desired nutrients and antioxidants.

Various tests were performed to analyse the nutritional value and its activity studies.Theywere as follows:

- Determination of moisture content in microgreens (90%) in radish microgreens
- Determination of solid weight in (10%) in radish microgreens.
- Phenol estimation in the given five samples grown in two respective growing medias i.e,cocopeat and VAM with highest pgenol in radish pink VAM microgreens (0.100mg/ml)
- Estimation of total flavanoid depictinh high TFC in Radish pink VAM microgreens (6.1mg/gf)
- Highest carbohydrate content in sunflower microgreens grown in cocopeat (1.633mg/ml)
- Highest protein content in sunflower microgreens (cocopeat) with a value of 4.9mg/ml by lowry’s method.
- Highest lipid profile observed in sunflower microgreens (cocopeat ) with 0.98%

- DPPH assay for Green mustard with highest TAC (0.21 mgGAE/g) showing 267.79$\mu$g/ml IC50 value.

In general, the microgreens investigated in this study were low in calories and fat but high in moisture content. Additionally, the microgreens contained relatively low carbohydrate and protein with the exception of mung beans and lentil microgreens, which had high carbohydrate (7.16 and 5.92 g 100 $g^{-1}$) and protein (4.55 and 6.47 g 100 $g^{-1}$, respectively) contents. Lentil microgreens had the highest total chlorophyll (112.62 mg 100 $g^{-1}$), carotenoid (28.37 mg 100 $g^{-1}$,), and ascorbic acid (128.70 mg 100 $g^{-1}$) contents. Buckwheat microgreens showed the highest TPC and maximum DPPH• scavenging activity. Only red cabbage and purple radish exhibited anthocyanin content, with the higher content found in red cabbage. The data provided in this study on microgreens of temperate and tropical origins will help farmers to select, expand, and add value to their business with the inclusion of microgreens.

Microgreens are more nutritious than vegetables with respect to some nutritionally important elements, but were able to qualify their statements by indicating that this would be highly dependent upon the growing method .In addition to requiring no fertilizer, analyses indicated that growing  microgreens on compost may require 220 to 328 times less water than . In addition to their relatively high nutritional value, microgreens may require smaller quantities of resources than industrially grown crops. Consuming microgreens could result in less waste, as people eat the entire plant, rather than eating onl florets  or leaves and throwing away the stems, as commonly occurs.

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# APPENDIX

## INSTRUMENTS

- Beakers
- Centrifuge
- Centrifuge tube
- Eppendorf tubes
- Flame photometer
- Glass rod
- Micropipettes
- Microtiter plates
- Mortar and pestle
- Refrigerator
- Spectrophotometer
- Test tubes
- Thermometer
- Water bath
- Weighing balance

## REAGENTS

- 0.1 N Sodium hydroxide
- 0.5% Copper sulphate
- 0.73% sodium chloride
- 1 M potassium acetate (CH3COOK)
- 2% Sodium carbonate

- 5% Ferric chloride
- 5% Phenol
- 7.5% sodium carbonate ($Na_2CO_3$)
- AluminiumChloride
- Bovine Serum Albumin (BSA)
- Chloroform
- Conc. Sulphuric acid
- Dil . Hydrochloric acid
- Distilled water
- DPPH (0.1 mM)
- Folin – Ciocalteau reagent
- Gallic acid
- Mayer’s reagent
- Methanol
- Molisch’s reagent
- Quercetin
- Deionised water