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Germination Timing, Plant Chemicals Screening and Assessment of Antioxidant Activity on Various Microgreens Vegetables Employing the DPPH (2,2-diphenyl-1-picrylhydrazyl) Technique

Hilmi Oktari, Warnita*, Ryan Budi Setiawan

Department of Agronomy, Faculty of Agriculture, Universitas Andalas, Padang 25163, West Sumatra, Indonesia

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Corresponding Author:
Warnita, Department of Agronomy, Faculty of Agriculture, Universitas Andalas, Padang 25163, West Sumatra, Indonesia
Email: warnita@agr.unand.ac.id

Abstract

The decline of agricultural land and the growth of urban areas have given rise to the idea of gardening in confined spaces. Urban agriculture is becoming more popular alongside the increasing recognition of healthy living. Microgreens are a type of vegetable that looks like sprouts but has a longer growth cycle, larger leaves, and a deeper green hue. These vegetables can be picked 7 to 21 days after planting. Nearly all varieties of microgreens contain much higher concentrations of bioactive compounds and antioxidants compared to fully grown leaves. This study aimed to assess the timing of germination, phytochemical composition, and antioxidant properties of microgreens. This research employed a fully randomized design (FRD) featuring a single treatment factor: microgreens, which encompassed green lettuce, red lettuce, green spinach, red spinach, and mustard greens. Data from observations were examined using a 5% variance test. The findings showed that gradual alterations in seed morphology mark germination phenology. Phytochemical analysis revealed the presence of tannins, saponins, flavonoids, phenolic compounds, and steroids. The greatest antioxidant activity was observed in mustard greens, boasting an IC50 value of 226.01 ppm. Thus, it can be determined that mustard greens possess the greatest antioxidant activity among microgreens.

Keywords: agriculture, antioxidant, phytochemical, microgreen, vegetables

1. Introduction

The idea of urban agriculture is becoming more popular as individuals gain a better understanding of the advantages of well-being practices. The diminishing quantity of arable land and the growing concentration of cities and settlements have led to the emergence of the idea of gardening in restricted areas. This idea is becoming more widespread within city populations and younger generations. One goal of urban farming is to ensure that the vegetables consumed are nutritious and pesticide-free, which supports a healthy lifestyle (Chrisnawati et al., 2022).Growing microgreens is one method to promote the The idea of city agriculture. Microgreens are vegetables similar to sprouts, but they have a longer growth period than sprouts, have larger, greener leaves, and can be harvested within 7–21 days after sowing (Ikrarwati et al., 2020). Microgreens cultivation demands knowledge of the best, premium-quality seeds that possess high viability and strength, which can be established via seed phenology research. Germination phenology examines the schedule of life-event processes and activities from seed to shoot. It provides information on the phases of plant germination (Leverett, 2017).

The secondary metabolite content of microgreens peaks before the plant enters the advanced growth phase, so harvesting at approximately 14 days of age yields the maximum concentration of bioactive compounds. Therefore, understanding germination phenology is crucial for determining the appropriate harvest time to obtain microgreens with optimal antioxidant activity and a representative phytochemical profile for laboratory analysis (Kyriacou et al., 2016).

The bioactive compounds and nutrients present in microgreens can be 4 to 40 times greater than those in fully developed plants. Furthermore, almost all types of microgreens contain significantly higher levels of bioactive compounds and antioxidants than mature leaves (Xiao et al., 2012). Numerous research papers have indicated the nutrient profile of microgreens. Xiao et al. (2016) investigated the mineral composition of 30 microgreen varieties representing 10 species across six genera in Plant Science. The research showed that potassium was the primary mineral, exhibiting concentrations between 176-387mg/100 g of fresh weight, followed by phosphorus (52-86mg/100 g fresh weight), calcium and magnesium (28-66mg/100 g of fresh weight), and sodium (19-68 mg/100 g of fresh weight)

Microgreens also contain phytochemicals with antioxidant properties that provide health benefits for the human body (Stefani & Andayani, 2022). Costless radicals are external substances that invade the body and may harm the immune system. Substances that can offset, remove, or counterbalance the impacts of free radicals are referred to as antioxidants. Antioxidants inhibit free radical reactions by donating electrons, thereby stabilizing reactive molecules and preventing oxidative chain reactions (Hasanah, 2015).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) test is a widely used method for assessing antioxidant content. The fundamental concept of the DPPH technique is a reaction in which the DPPH free radical accepts a hydrogen atom from an antioxidant, and absorbance is measured at the peak wavelength with a spectrophotometer. Antioxidant activity in a sample is indicated by a color change in the DPPH solution in methanol, from initially deep purple to pale yellow (Hidayat et al., 2017).

This research sought to identify the germination phenological stages, antioxidant properties, and phytochemical characteristics of various microgreens.

2. Materials and Methods

This study was carried out at the Tissue Culture. Laboratory and Plant Physiology Lab, Faculty of Pertanian, Universitas Andalas, Padang, and the examination was carried out at the Higher Education Service LLDIKTI Institute Region 10 Lab, Padang, in July-August 2025.

The seed utilized were green lettuce seeds of the Grand Rapids variety, red lettuce seeds of the Red Rapids variety, green spinach seeds of the Maestro variety, red spinach seeds of the Mira variety, mustard seeds of the Shinta variety, compost (P4S Sungkai Permai), H2O, distilled water, label paper, duct tape, masking tape, tissue, aluminum foil, hydrochloric acid (HCl), 96 % ethanol (C2H5OH), Whatman paper no. 1, iron (III) chloride (FeCl3) 1%, sulfuric acid (H2SO4), Wagner reagent, acetic acid (CH3COOH), methanol p.a., vitamin C (C6H8O6), and DPPH (2,2-diphenyl-1-picrylhydrazyl).

The equipment utilized comprised plastic seedling trays, digital scales, precision scales, shelves, a spray bottle, shears, a white LED light bulb, wires, a timer outlet, dark plastic, a microscope, an oven, a blender, a measuring cylinder, a 60-mesh sieve, a centrifuge, a spectrophotometer, a vortex mixer, test tubes, a volumetric flask, pipettes, a micropipette, a rotary evaporator, a stirring rod, a glass bottle, a drip plate, a ruler, documentation tools, and stationery.

This experiment was set up in a Completely Randomized Design (CRD) with five microgreen varieties: green lettuce, red lettuce, green leafy greens, red leaf spinach, and mustard leaves. Each treatment level was repeated 3 times, resulting in 15 experimental subjects.

Results of the phytochemical tests were provided descriptively, while IC50 data were analyzed using the Statistical Tool for Agricultural Research (STAR). If the probability was less than 0.05, Duncan's New Multiple Range Test (DNMRT) was used at the 5% level.

3. Results and Discussion

Seed Germination Phenology

Germination phenology refers to the progressive changes in the structural characteristics of young plants. Germination phenology is an important factor in gauging the potential for seedling establishment and initial plant growth. Germination duration is frequently used as a signal or predictor to assess whether specific seeds can effectively support initiatives aimed at enhancing seedling quality. Plants are said to be growing when changes in appearance occur, for example, during the vegetative phase, changes begin with germination, followed by the emergence of seedlings above the soil, leaf formation, and root formation (Cahyani et al., 2023). The phenological phases of microgreen seed sprouting, noted as 0-14 days post-germination, are illustrated in Figure 1.

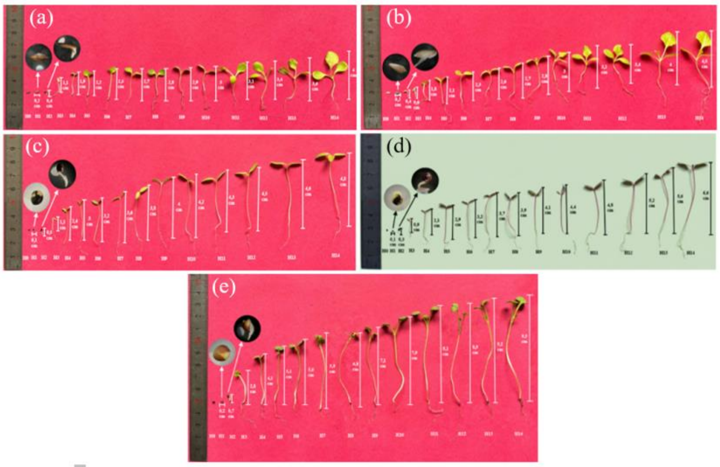

Figure 1. Phenology of seed germination aged 0-14 days, a) green lettuce, b) red lettuce, c) green spinach, d) red spinach, e) mustard greens.

Lettuce, spinach, and mustard greens are categorized as orthodox seeds, meaning they can withstand extended storage by reducing moisture to ±5–7% while maintaining viability and are resistant to low temperatures and freezing. Orthodox seeds are characterized by their resistance to desiccation, preserved viability during refrigeration, small to medium size, and an extended shelf life of up to several years under the right conditions. This resistance is due to the seeds' ability to undergo natural desiccation during the final stage of maturation, characterized by the accumulation of sugars such as trehalose and sucrose, as well as protective proteins (LEA proteins) that protect cell membranes and enzymes from damage during drying (Walters, 2015).

Germination marks the beginning of plant development, with the radicle appearing after imbibition. The germination process begins with water absorption, which activates metabolism, increases respiration, and breaks down food reserves. Imbibition causes the embryonic tissues to swell, allowing the radicle to break through the seed coat. Haq et al. (2024) stated that the main biochemical change during the germination phase is increased energy (ATP) production from aerobic respiration, which is used for enzyme synthesis and other physiological activities. Hormonal activation, particularly gibberellin (GA), plays an essential part in breaking dormancy and stimulating the synthesis of hydrolytic enzymes like α-amylase, which breaks down starch into simple sugars for energy. Furthermore, protease and lipase enzymes hydrolyze protein and lipid stores to generate amino acids and fatty acids, which are sent to the developing.

This is supported by Pompelli et al. (2023), who reported that water absorption activates embryonic cells and accelerates germination. Water can enhance cellular respiration to generate ATP, thin the protoplasm, activate metabolism, and act as a medium for nutrient transport from the endosperm or cotyledons to the growing point. The process of imbibition, or water absorption in seeds, softens the seed coat, releasing the layer and allowing the embryo to germinate.

The first organ to develop once the seed absorbs water is the root, which elongates and produces root hairs. The radicle is the part of the embryo located at the base and functions as the plant's root (Yora et al., 2024). The radicles of microgreens such as green lettuce, red lettuce, green spinach, red spinach, and mustard greens emerge on the second day after planting, measuring 0.4 cm, 0.4 cm, 0.5 cm, 0.3 cm, and 0.7 cm, respectively. Variations in radicle length the following day across all varieties of vegetables can be influenced by seed genetics and physiology. The longest roots in mustard greens may result from their larger size and seed reserves, allowing them to grow longer in a shorter time.

The next stage of germination is the emergence of the hypocotyl. The hypocotyl refers to the section of the embryonic stem located between the root tip (radicle) and the cotyledons in seedlings after imbibition. Hypocotyl emergence was noted on the third day after sowing in all types of vegetables. Hypocotyl growth involves apical meristem activity and cell elongation, influenced by hormones such as auxin and gibberellin (GA), as well as environmental conditions such as light, temperature, and humidity (Hasan et al., 2023).

The hypocotyls of mustard, spinach, and lettuce greens elongate, elevating the cotyledons above the soil surface, suggesting that germination in all microgreen vegetable seeds is epigeal. The hypocotyl is crucial in the germination process, particularly in the epigeal type, where it stretches to draw the cotyledons emerging from the soil. The phase of lifting and opening cotyledons in green and red lettuce, green spinach, and red spinach appeared on the fourth day following planting. The phase of lifting and opening cotyledons in green and red lettuce, green spinach, and red spinach appeared on the fourth day following planting. In mustard greens, cotyledons rose and spread open concurrently with hypocotyl emergence, observed on the third day following planting.

The hypocotyl continues to elongate during germination, and afterward, the plant undergoes the initial leaf emergence stage. The moment when the first authentic leaf appears varies across therapies. It can be ordered from quickest to the slowest: mustard greens at 8 days post-planting, red lettuce at 10 days post-planting, green lettuce at 11 days post-planting, green spinach at 12 days post-planting, and red spinach at 14 days post-planting. This reflects variation in physiological, genetic, and seed vigor. The duration until the initial leaf appears in mustard greens is typically shorter due to larger seed size and greater food reserves, thereby supporting embryo growth and tissue differentiation.

Phytochemical Screening (Qualitative)

Phytochemical screening consists of preliminary qualitative tests used to detect secondary metabolites in plant extracts. The primary objective of phytochemical screening is to provide an initial overview of the secondary metabolites that could contribute to a plant's biological activity, such as antioxidant activity (Kalemba et al., 2024).

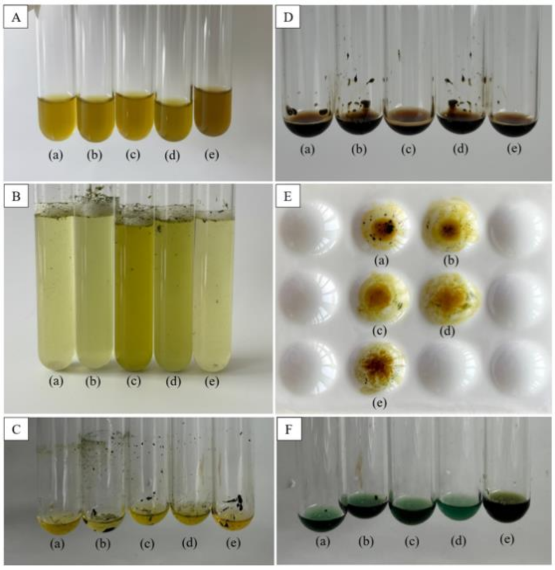

Figure 2. Changes in the color of the test solution, A) tannin, B) saponin, C) alkaloid, D) flavonoid, E) phenolic, F) steroid, a) green lettuce, b) red lettuce, c) green spinach, d) red spinach, e) mustard greens.

Phytochemical screening is important in pharmacological and health research because secondary metabolites often act as key bioactive compounds. For example, flavonoids are known to exhibit high antioxidant activity by donating electrons to neutralize free radicals, while saponins can act as immunomodulatory and antimicrobial agents (Ezeonu & Ejikeme, 2016).

a. Tannin

Tannins are secondary metabolites naturally synthesized by plants and generally accumulate in cell walls or vacuoles, especially in roots, shoots, leaves, stems, and seeds. These compounds are widespread in gymnosperms and angiosperms, but are most abundant in dicotyledonous plants (Hersila et al., 2023). Tannin screening results for several microgreens revealed the presence of tannins in green lettuce, red lettuce, green spinach, red spinach, and mustard greens (Figure 2A).

b. Saponin

Saponins are secondary metabolites frequently present in plants that produce a stable foam when dissolved in water and shaken. Chemically, saponins belong to the glycoside group, which are compounds composed of sugar molecules (glycones) bound to a non-sugar part (aglycone) called sapogenin. These sapogenins can be triterpenoids or steroids, leading to variations in the physical properties and biological activities of saponins across different plant species (Darma et al., 2020). Phytochemical screening revealed the presence of saponin in all tested microgreen samples. This was indicated by the formation of a stable foam in the test solution (Figure 2B) that persisted standing for 5 minutes, consistent with a positive saponin reaction.

c. Alkaloid

Alkaloids are a large group of secondary plant compounds frequently present in plants and generate persistent metabolites with heterocyclic structures that contain nitrogen atoms. Most alkaloids are naturally alkaline and readily react with acids to form salts. (Silla et al., 2020). Phytochemical screening for alkaloids in several types of microgreens yielded negative results. This was demonstrated by the test solution's failure to form a precipitate upon addition of Wagner's reagent, suggesting that alkaloid compounds were not present in the samples (Figure 2C).

d. Flavonoid

Secondary metabolites that fall under the category of polyphenolic compounds are called flavonoids. The fundamental structure of these molecules is an aromatic ring with one or more hydroxyl groups, which is produced by combining two primary biosynthetic pathways: phenylpropanoids and polyketides. Fruits, vegetables, and nuts are common sources of flavonoids. Flavonoids are pigments found in plants that give flowers and fruits yellow, red, or purple hues to attract pollinators. Fruits, vegetables, and nuts are common sources of flavonoids. Flavonoids are pigments found in plants that provide flowers and fruits with yellow, red, or purple hues to help attract pollinators. Additionally, flavonoids shield plants from environmental stresses like oxidative stress, pathogen invasion, and UV radiation. In humans, flavonoids are known to have various pharmacological benefits, including antioxidant, anti-inflammatory, and antimicrobial properties, as well as potential roles in preventing degenerative diseases such as cancer and cardiovascular disease (Azalia et al., 2023).The sample solution's color changed as a result of the phytochemical test (Figure 2D), indicating a positive reaction to flavonoids.

e. Phenolic

A class of secondary metabolites known as phenolic compounds is distinguished by an aromatic ring structure that contains one or more hydroxyl groups. These compounds are commonly found in plant tissues, including vegetables. Common phenolic compounds found in vegetables include phenolic acids (chlorogenic acid, ferulic acid, caffeic acid), flavonoids (quercetin, kaempferol, anthocyanins), and tannins.

When $FeCl_3$ reagent was added, phytochemical assays for phenolic compounds revealed a color shift in the sample solution, indicating a positive reaction to phenolic compounds (Figure 2E).

f. Steroid

Secondary metabolites of the triterpenoid group, steroid chemicals, are crucial to plants as membrane components and growth regulators. When the reagent was added, the sample solution's color changed, indicating a positive reaction in the phytochemical assays for steroid chemicals. (Figure 2F).

g. Antioxidant Activity

Using a UV-Vis spectrophotometer, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is frequently used to assess antioxidant activity in plant extracts, including microgreens. The highest absorption of DPPH, a stable, dark purple free radical, occurs at a wavelength of roughly 517 nm.

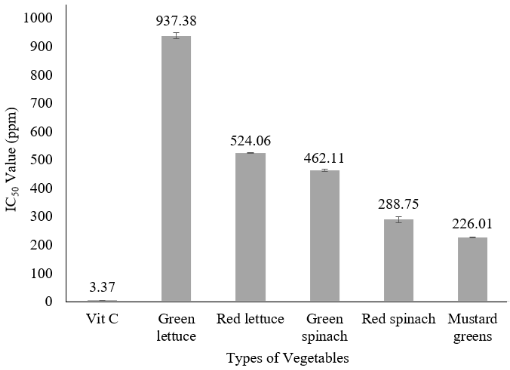

Figure 3. Graph of average IC50 values of microgreens

Microgreen extracts from lettuce, spinach, and mustard greens showed variable free radical-scavenging capacity, as determined by antioxidant activity assays using the DPPH method (Figure 3). The IC50 value, i.e., the extract concentration required to reduce DPPH radicals by 50%, was used to measure antioxidant activity. As a reference, the well-known strong antioxidant, ascorbic acid (Vitamin C), was used. Ascorbic acid's IC50 value of 3.37 mg/L demonstrated extremely potent antioxidant activity. Microgreen extracts showed relatively little antioxidant activity when compared to ascorbic acid, with mustard greens, red spinach, green spinach, red lettuce, and green lettuce showing the highest to lowest antioxidant activity.

Several microgreens' average IC50 values. All of the samples had extremely high IC50 values, all above 200 ppm. However, mustard greens had the lowest IC50 (226.01 ppm), followed by red spinach (288.75 ppm), green spinach (462.10 ppm), red lettuce (524.06 ppm), and green lettuce (938.38 ppm). Since microgreens are harvested during the early growth period, roughly 7–14 days after sowing, their relatively low antioxidant activity may be attributed to their developmental stage. The relatively low antioxidant activity observed in microgreens may be attributed to their developmental stage, as they are harvested during the early growth phase, approximately 7–14 days after sowing, resulting in relatively low accumulation of secondary metabolites such as phenolics, flavonoids, triterpenoids, and steroids, which act as antioxidant compounds (Kyriacou et al., 2016). Additionally, several antioxidant compounds—triterpenoids and flavonoids in particular—tend to be concentrated in the cuticle wax layer or in specialized organs like roots and stems. Young microgreen leaves have thin cuticles and underdeveloped root systems, leading to lower secondary metabolite content than in mature plants (Yang et al., 2018).

4. Conclusions

The germination phenology of the assessed microgreen species varied and was observed 1–14 days after seeding, according to the study's findings, beginning with cotyledon lifting and opening, radicle emergence, hypocotyl emergence, hypocotyl growth, first leaf emergence, and first leaf color. Tannins, saponins, flavonoids, phenolics, and steroids are among the phytochemicals found in mustard greens, green spinach, red spinach, and red lettuce. Mustard greens have the highest antioxidant activity among microgreens.

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