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PHYTOCHEMICAL PROFILE AND SILVER NANOPARTICLE SYNTHESIS OF MICROGREENS (VIGNA RADIATA AND VIGNA UNGICULATA)

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ABSTRACT

This study investigates the phytochemical composition and green synthesis of silver nanoparticles (AgNPs) using microgreens of Vigna radiata (Mung bean) and Vigna unguiculata (Red cowpea). Microgreens were cultivated, harvested, shade-dried, and subjected to ethanolic extraction. Phytochemical screening confirmed the presence of flavonoids, carbohydrates, proteins, and saponins. The extract was utilized for silver nanoparticle synthesis, which was characterized using scanning electron microscopy (SEM). The antimicrobial activity of AgNPs was tested against Escherichia coli and Bacillus sp., showing significant inhibition zones. These findings highlight the potential of microgreens as a sustainable source for nanoparticle synthesis with biomedical applications.

KEYWORDS: Microgreens, Phytochemicals, Silver Nanoparticles, *Vigna radiata*, *Vigna unguiculata*, Antimicrobial Activity.

INTRODUCTION

Microgreens

Microgreens have explicit well-being advancing and infection forestalling properties, and also considered as practical nourishments. Deficiency of proteins as well as other wholesome lack of minerals can be tackled by the arising class of microgreens. Edible young greens are

produced from various kind of vegetables, herbs, and other plants. They range in size from 1 to 3 inches (2.5 to 7.6cm), including the stem and leaves. The stem is cut just above the soil line during harvesting. Microgreens have fully developed cotyledon leaves and usually, one pair of small, partially developed true leaves. Microgreens are young and tiny seedlings of vegetables and herbs which usually grows within 7 to 14 days after germination, which varies from crop to crop from variety to variety and other environmental conditions. It has 3 basic parts; a central stem, 2 cotyledon leaves and typically the first pair of very young true leaves.

Microgreens are cut along with the stem and attached cotyledons/ seed leaves with the help of scissors if left for longer time, they will begin to rapidly elongate and lose colour and flavour. Microgreens are different from any other small plants like baby greens in their size and in their properties. They are also known as vegetable confetti.[1]

Mung bean

Mung bean plant is used as a source of protein. Mung bean plant also known as *Vigna radiata,* is widely consumed across the world and holds a significant place in human nutrition due to its rich protein content and various active compounds. Containing between 20.97% and 32.6% protein, mung bean sprouts are an excellent source of high-quality proteins, particularly amino acids that are essential for human health. This protein content is particularly beneficial because it includes essential amino acids that many cereals lack, making mung beans plants a valuable addition to diets that rely heavily on grains. As a result, mung beans leaves are often used as a staple food in countries like India and Pakistan, where they are commonly prepared as "dhal." Dhal, a popular legume-based dish, meets the daily protein needs of the population, providing not only sufficient protein but also ensuring its bioavailability, meaning the nutrients are easily absorbed and utilized by the body.

In addition to protein, mung bean plants are packed with essential nutrients such as minerals (including iron), dietary fibre, and significant amounts of bioactive phytochemicals, making them a functional food that offers numerous health benefits. These nutrients support various aspects of human health, from improving digestion to preventing nutrient deficiencies. Iron, for example, is crucial in the formation of red blood cells, which helps prevent anaemia, while dietary Fibre contributes to better digestive health and helps regulate blood sugar levels.

Moreover, mung beans plants are rich in compounds such as polyphenols, polysaccharides, and polypeptides, all of which contribute to their antioxidant properties. Antioxidants play a vital role in neutralizing harmful free radicals in the body, reducing oxidative stress, and preventing cellular damage. This makes mung beans particularly useful in preventing or managing chronic diseases such as diabetes, heart disease, and certain types of cancer. Several studies have highlighted the potential health benefits of mung beans, including their hypoglycaemic (blood sugar-lowering), hypolipidemic (cholesterol-lowering), and antihypertensive (blood pressure-lowering) effects.

Additionally, mung beans leaves have shown promising results in protecting the liver (hepatoprotective activity), enhancing immune function (immunomodulatory effects), and even inhibiting the formation of melanin, which can help with skin conditions (anti-melanogenesis). Their ability to support liver health and modulate the immune system adds to their appeal as a functional food that promotes overall well-being.

The bioactive compounds found in mung beans plants, such as phenolic acids, flavonoids, and peptides, also contribute to their anti-cancer properties. These compounds have been studied for their potential to inhibit the growth of cancer cells and prevent the development of tumours. While many of these health benefits have been demonstrated in both in vitro and animal studies, further research is ongoing to better understand the full potential of mung beans in human health.[2]

Here's a brief overview of the characteristics and activities of the stem and leaf of a mung bean Stem:

Characteristics

1. Upright/vine growth habit
2. 0.5-1.3m length
3. Green, smooth, round

Activities

Supports plant structure

1. Transports water, nutrients, sugars
2. Produces auxins (Growth hormones)
3. Anchors leaves for photosynthesis

Leaf

Characteristics

1. Trifoliate (3 leaflets)
2. Alternate arrangement
3. Ovate/lanceolate shape
4. 5-15cm long, 3-7cm wide
5. Green, smooth

Activities

1. Photosynthesis (Food production)
2. Transpiration (Water regulation)
3. Gas exchange (O2, CO2)
4. Synthesis of amino acids, proteins

Key functions

1. Stem: Provides structural support, facilitates transport
2. Leaf: Produces food through photosynthesis, regulates water.[21]

The taxonomy of *Vigna radiata*, commonly known as mung bean or green gram, is well-defined within the plant kingdom, as follows:

1. **Kingdom:** Plantae

This kingdom includes all living plants, including trees, herbs, shrubs, and more.

2. **Subkingdom:** Tracheobionta (Vascular plants)

These are plants with specialized tissue (xylem and phloem) for transporting water and nutrients.

3. **Superdivision:** Spermatophyta (Seed plants)

This division encompasses plants that reproduce through seeds, as opposed to spores.

4. **Division:** Magnoliophyta (Angiosperms or flowering plants)

Angiosperms are seed-producing plants that bear flowers and fruit.

5. **Class:** Magnoliopsida (Dicotyledons)

This class consists of dicotyledons, plants whose seeds contain two embryonic leaves (cotyledons). Dicots typically have broad leaves with reticulate venation.

6. **Subclass:** Rosidae

A Large group within dicotyledons that includes many species of flowering plants.

7. **Order:** Fabales

This order includes leguminous plants, which are known for their ability to fix nitrogen, making them important for soil health and agriculture.

8. **Family:** Fabaceae (Leguminosae or legume family)

One of the largest families of flowering plants, Fabaceae includes beans, peas, and other legumes, many of which are staple crops due to their high protein content and nitrogen-fixing properties.

9. **Genus:** *Vigna*

A Genus that includes various species of beans and pulses, commonly cultivated in tropical and subtropical regions for their edible seeds.

10. **Species:** *Vigna radiata*

The specific species, commonly known as mung bean, is recognized for its small, green seeds and its significant role as a food source due to its high nutritional value, particularly in protein and micronutrients.[19]

Red cow peas

Cow pea is a warm-season plant. It is an annual herbaceous legume. It is a drought resistant plant. Like any other legumes cow pea plant have high nutritional value. They contain high amount of protein. They incorporate lot of several phytochemicals in their plant profile. Red cow pea is the largely cultivated and commonly consumed in the old world (ALLEN and ALLEN 1981).

Vigna unguiculata, commonly known as cowpea, belongs to the following taxonomic classification:

Kingdom: Plantae

Subkingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Fabales

Family: Fabaceae (Formerly leguminosae)

Genus: *Vigna*

Species: *Vigna unguiculata.*

The genus Vigna was established by Savi in 1824 and is dedicated to Domenico Vigna, a professor of botany in Pisa, Italy. This genus includes over 100 wild species and is divided into seven subgenera. Among these, the recognized subgenera that have yielded economically important crop species include Ceratotropis, Plectrotropis, and Vigna. Cowpea is part of a group of herbaceous legumes that thrive in warm climates, although they do not survive winter in temperate regions. This species is linked to the genus Phaseolus, which encompasses more than 20 species native to tropical areas of the New World. The economic significance of *Vigna unguiculata* is notable in developing countries, where it is cultivated for its nutritional and medicinal properties.[3]

Cow pea plant is an introduced species in United States. Nodules on the cow pea roots fix nitrogen fixation in soil. Varieties may be shorty and bushy, prostrate, or tall and vine like. Canopy can be of 23 inches depending on the variety of cow peas. Red cow peas can invariably grow both in cultivated and in wild. THOMAS JEFFERSON introduced the name cow peas. Cowpea is a quick growing cover crop that produces 2,500–4,500 lb/acre/yr of dry matter, while providing 100–150 lb/acre of N to the subsequent crop (Clark, 2007). Its long taproot and wide, vegetative spread make it an excellent plant for erosion.

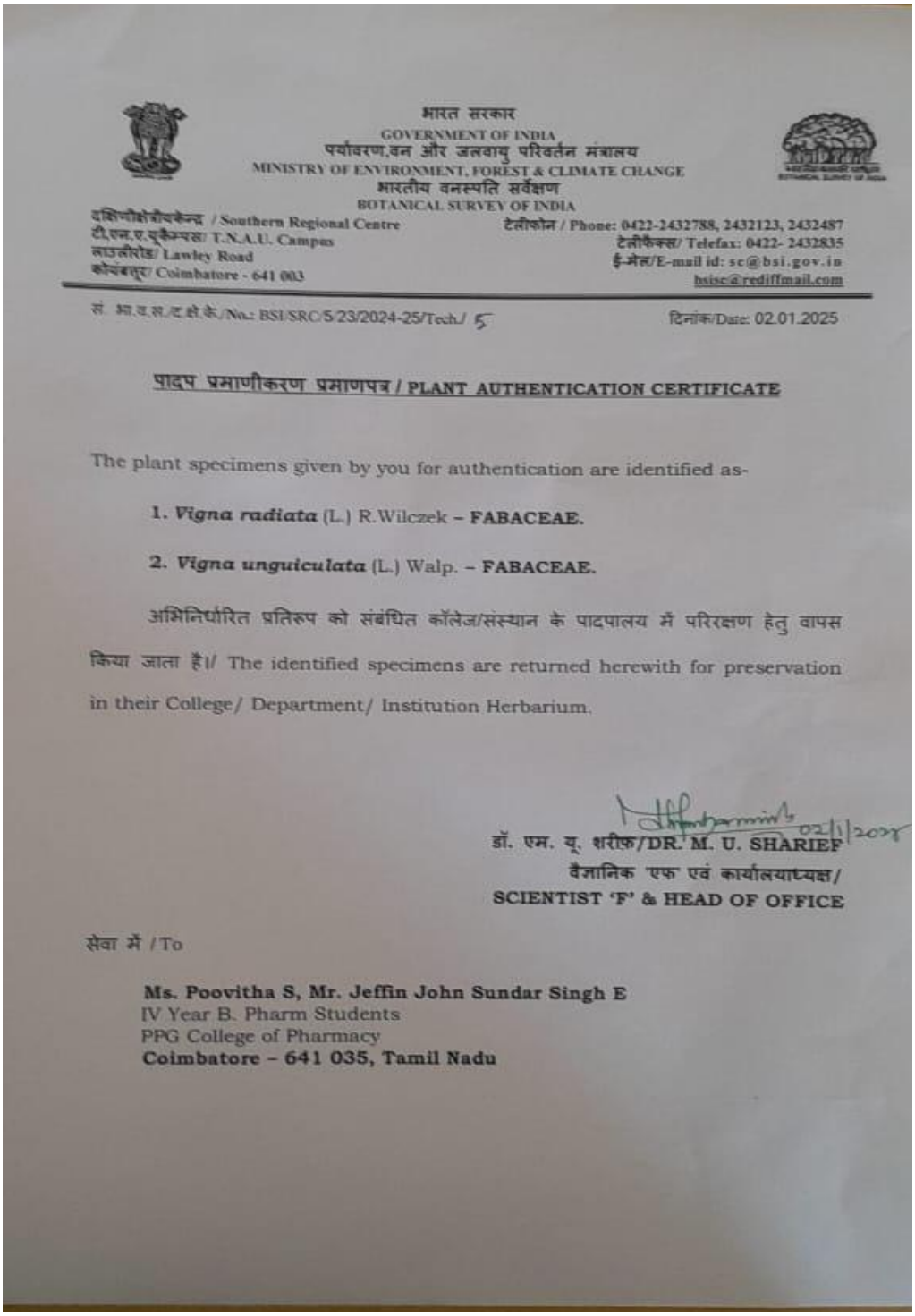
Cowpea (*Vigna unguiculata*) plays a significant role in wildlife nutrition and habitat. It serves as a valuable forage crop for deer, making it a popular choice for food plots aimed at attracting these animals. The tender leaves and nutritious seeds are particularly appealing to deer, promoting their health and well-being. Additionally, various bird species, including wild turkey, feed on cowpea seeds, taking advantage of this nutritious food source. The plant also provides essential cover for quail and other ground-nesting birds, helping them evade predators. Some cowpea varieties are specifically cultivated to enhance wildlife habitats, demonstrating their ecological importance.[15]

The plant of *vigna unguiculata* has many properties they are associated with inhibitory effect in the reduction of coronary heart disease.[4]

The seeds of *Vigna unguiculata*, commonly known as cowpea, exhibit a distinctive kidney shape that plays a significant role in its identification and classification. Both wild and domesticated varieties of this plant showcase a unique maculo-reticulate pattern on their seed coats, which is characterized by a network of small dots and lines that contribute to their visual appeal and differentiation. The coloration of the seeds varies significantly between wild and cultivated forms; wild cowpea seeds are typically black, while domesticated seeds often present a creamy hue.

The seeds are reniform, meaning they are kidney-shaped, and they are compressed, measuring approximately 5 to 6 mm in length, 3 to 4 mm in width, and 2 to 3 mm in thickness. The seed coat is not only lustrous but also firm, providing protection to the delicate embryo inside. A notable feature of the seeds is the hilum, which is the scar left from the attachment of the seed to the ovary wall. The hilum measures about 1 to 1.5 mm in length and is situated close to the micro Pyle, which is the opening that allows for the entry of water during the germination process.

Importantly, Vigna unguiculata seeds are classified as non-endospermic, meaning they do not contain a food storage tissue typically found in other seeds. Instead, they rely on the cotyledons for nourishment during the initial stages of germination. The plant itself has a tough yet thin seed coat, except for the hilum region, which is relatively softer, allowing for efficient moisture absorption. The seeds are classified as fleshy dicotyledons, with each seed containing two seed leaves, or cotyledons, that are approximately 5 to 6 mm in length and 4 to 5 mm in width, along with an incurved radical measuring about 4 mm. This unique structure and composition of the seeds make cowpea an essential crop in many agricultural practices worldwide.[3]



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सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2024-25/Tech/ 5

दिनांक/Date: 02.01.2025

पादप प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The plant specimens given by you for authentication are identified as-

1. ***Vigna radiata*** (L.) R.Wilczek – **FABACEAE.**
2. ***Vigna unguiculata*** (L.) Walp. – **FABACEAE.**

अभिनिर्धारित प्रतिरूप को संबंधित कॉलेज/संस्थान के पादपालय में परिरक्षण हेतु वापस किया जाता है।/ The identified specimens are returned herewith for preservation in their College/ Department/ Institution Herbarium.

02/1/2025

डॉ. एम. यू. शरीफ/DR. M. U. SHARIEF
वैज्ञानिक 'एफ' एवं कार्यालयाध्यक्ष/
SCIENTIST 'F' & HEAD OF OFFICE

सेवा में / To

Ms. Poovitha S, Mr. Jeffin John Sundar Singh E
IV Year B. Pharm Students
PPG College of Pharmacy
Coimbatore – 641 035, Tamil Nadu

Fig. 1: Authentication certificate.

MATERIALS AND METHODS

Plant growth

Microgreens can be grown outside as well as inside and that is the irony part of growing micro greens because deciding whether to grow it inside or outside is first and foremost factor. Since growing microgreens inside is promotes more prominent growth over the

fluctuating and developing climate. Micro greens do not require any additional nutritional requirements for their growth but they do need appropriate environmental conditions. The main distinction between sprouts, microgreens, and baby greens (Later stage of microgreens) is the size of the plant and growing time.[10]

Firstly, the microgreens are germinated using a cloth. If the seeds are germinated in a condition provided with 12 h/12 h light/dark period and the temperature should be around 28 degrees Celsius.[15] The green gram as well as the red cow peas are purchased in an adequate amount then, they are rinsed in distilled water and drained. Then they are placed in distilled water and soaked overnight in the dark for 8 hours at (25±3°C). After pouring off the soaking water, the seed were rinsed in water for about 5 minutes and then the seeds are covered by cloth and kept for the whole night or can be spread evenly on an adsorbent paper and then placed in a controlled environment chamber at 28 degrees Celsius. The seeds were showered with water once a day and left to drain and the germinated seeds were collected at the end of the treatment.[14]

Now the germinated seeds are collected separately then they are sown in plastic trays which contain soil. The germinated seeds were sprayed with water twice and were kept inside the room near the window.

Harvesting

After the seeds have germinated, they will be allowed to grow for one week to achieve optimal development. At harvest time, 3-4 inches grown plants will be carefully plucked from the soil, ensuring that the surrounding environment is minimally disturbed to prevent contamination. During this process, a sterilized blade will be utilized to cut off the roots, separating them from the aboveground portions of the plants. This careful handling is essential to preserve the integrity of the samples for subsequent analysis. The harvested plants will then be prepared for further study, including extraction and estimation of phytochemical constituents present in *Vigna radiata and Vigna unguiculata*.

Drying

After harvesting germinated sprouts are subjected to shade drying for 2 days.[18] In shade drying the plant that should be dried should not be exposed to sun light to avoid ultra violet rays, which can break down the sensitive compounds such as chlorophyll and essential oils.

Regular monitoring is essential for proper drying of the microgreens. The material becomes fully dry when it gets brittle.[19]

Mung bean

Fig. 2: Mung bean picture.

Red cow peas

Fig. 3: Red cow peas picture.

Extraction procedure

Extraction definition

In pharmacognosy, extraction refers to the process of isolating active compounds from natural sources, particularly plants. This involves using solvents to dissolve and separate bioactive phytochemicals, such as alkaloids, flavonoids, and terpenoids, which can have therapeutic effects. Various techniques are employed, including maceration, percolation, and Soxhlet extraction, with the choice depending on the properties of the target compounds. Effective extraction is essential in pharmacognosy to study, identify, and utilize these compounds for medicinal purposes.

Extraction procedure

The extraction method chosen for isolating these compounds is critical, as it significantly influences.[23] After drying, the microgreens are subjected to extraction process. The method used here is maceration extraction process using ethanol.

The crude powder of both the microgreens are combined and is kept aside in a separate container. Then 5 gram of the powder is taken in a beaker then 100ml of ethanol (96 percent) is poured into the beaker. Then the contents are mixed by through agitation and shaking for 10 minutes.[21]

The beaker is kept undisturbed for 24 hours and thus they are stored at room temperature. The extract will also be utilized to determine the total phenolic content, providing insights into its phytochemical profile. This analysis will be crucial for understanding the potential health benefits associated with the phenolic compounds present in the extract.[22]

Qualitative estimation of phytoconstituents

Vegetables are important in the diet to prevent and control diseases. According to WHO insufficient fruit and vegetable intake is likely to cause 2.7 million deaths all around the world. Some microgreens contain more phytoconstituents than the other comparatively. However exact quantity of microgreens is not clearly stated. All microgreens contain its own amount of nutrition and phytoconstituents. Most of the phytoconstituents present are phyllo Quinone, carotenoids, and polyphenols. Species such as potatoes, tomatoes, egg plants, peppers and potentially rhubarb are toxic if eaten when immature (Jagatheeswari, 2014). The consumption of these plants might produce several adverse effects like headache, vomiting and many other several diseases. These phytoconstituents play a vital and major role in the research into cancer prevention. It is suggested that a third of all cancers could be prevented by eating diet rich in fruits and vegetables and low in fat and calories (Donaldson, 2004; Betoria et al., 2016).[7]

Germinating seeds have been shown to contain between 2 to 10 times more phytochemicals compared to their commercial adult counterparts. The concentration of these beneficial compounds can vary significantly based on several factors, including the species, cultivar, environmental conditions, and the duration of germination, storage, and processing. While sprouts have often received attention as health-promoting foods, microgreens are also emerging as valuable dietary additions due to their rich nutrient and bioactive compound

content. Both sprouts and microgreens are increasingly recognized and recommended by dietitians for their high levels of flavonoids, hydroxycinnamic acids, vitamins, glucosinolates, minerals, and carotenoids.

Phytochemicals present in these young plants play a vital role in protecting the human body against various chronic disorders, including cardiovascular diseases, diabetes, and certain types of cancer. For instance, flavonoids are known for their antioxidant properties, which can help reduce inflammation and oxidative stress, contributing to overall health. Moreover, the caloric value of sprout and microgreen leaves is relatively low, ranging from 29 to 128 kcal per 100 grams, which is beneficial for those seeking to maintain a healthy weight or manage their caloric intake.[8]

1. Test for alkaloids

The extracts of microgreens were warmed separately with 2% H2S04 for 2 minutes. It was filtered and few drops of following reagents were added, which indicated the presence of alkaloids Dragendroff's reagent: A red precipitation indicated the positive test. Mayer's reagent: A creamy white colored indicated the positive test. Picric acid: A yellow precipitation indicates the positive test.

2. Test for flavonoids

A small quantity of the extract was heated with 10ml of ethyl acetate in boiling water for 3mins. The mixture was filtered and the filtrates were used for the following tests. The filtrate was shaken with 1ml of dil. Ammonia solution (1%). The layers were allowed to separate. A yellow coloration was observed in ammonia layer indicating the presence of flavonoid. The filtrate was shaken with 1ml of 1% Ammonium Chloride solution, where light yellow color was observed. It indicated the presence of flavonoid.

3. Test for carbohydrates

The extracts were shaken vigorously with water and filtered. A few drops of Molisch's reagent was added to the aqueous filter, followed by vigorous shaking again. Concentrated H2SO4 (1ml) was carefully added to form a layer below the aqueous solution. A brown ring at the interface indicated the positive test.

4. Test for saponins

A small quantity of different extracts was diluted with 4ml of distilled water. The mixture was shaken vigorously and then observed on standing for stable brake, which indicated positive test.

5. Test for steroids

2 ml of Acetic anhydride and 2ml H2SO4 were added to the extracts. The color changed from violet to blue or green, which indicated the presence of steroids.

6. Test for Anthraquinone glycosides (Born Trager's test)

Dil.H2SO4 was added to the extracts and boiled. Then filtered and cooled. To the cold filtrate, 3 ml of benzene was added and mixed. The benzene layer was separated and ammonia (2ml) solution was added to it. A rose pink to red color in ammoniac layer was observed, which indicated positive test.

7. Test for cardiac glycosides (Legal's test)

To each extract, 1ml of pyridine and 1ml of sodium nitroprusside solution were added and observed. A deep red color was observed indicating the positive test.

8. Test for terpenoids (Salkowski test)

Each extract was mixed with 2ml of chloroform and then concentrated H2SO4 (3ml) was carefully added to form a layer. A reddish brown coloration at the interface indicated positive result for the presence of terpenoids.

9. Test for Gum and Mucilage

Each extract was dissolved in 10ml of distilled water and 25 ml of absolute alcohol was added to it with constant stirring. White or cloudy precipitate indicated the presence of gum and mucilage's.

10. Test for Amino Acids and Proteins

Each extract was dissolved in 10 ml of distilled water and the filtrate was subjected to test the presence of proteins and amino acids.

11. Ninhydrin test

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) were added to 2ml of aqueous filtrate. A characteristic purple color indicated the presence of amino acids.

Quantitative estimtion of protien

Over the years, various methods have been developed to quantify protein content in samples. Traditional techniques, such as gravimetric methods for lyophilized (freeze-dried) proteins, have been used alongside elemental analysis methods like the Kjeldahl nitrogen assay, which has been a staple since 1983. However, these methods are becoming less common as researchers increasingly turn to colorimetric and spectroscopic techniques. These modern methods offer several advantages, including convenience and reasonable accuracy when estimating protein content in solution.

Colorimetric assays, such as the Bradford and Lowry methods, rely on specific dye-binding reactions with proteins, producing a colour change that can be measured spectrophotometrically. Spectroscopic methods, including UV-visible spectroscopy, utilize the absorbance of proteins at specific wavelengths to determine concentration. These techniques are widely favoured due to their speed and ease of use, making them suitable for various laboratory settings.

In addition to these methods, there are comprehensive reviews available for those interested in exploring alternative protein quantification techniques, such as the one by Darbre in 1986. A recent comparative analysis of common methods for protein concentration analysis has also been published by Bio-Rad Laboratories, providing valuable insights into the effectiveness and application of these techniques.[11]

Total Phenolic Content (TPC)

Method: Folin-Ciocalteau method (Vats, 2012)

Steps

1. Add 0.5 mL of distilled water and 0.125 mL of the sample to a test tube.
2. Add Folin-Ciocalteau reagent, sodium carbonate solution, and distilled water successively.
3. Allow the mixture to stand for 90 minutes.
4. Measure the absorbance at 760 nm.

TPC is expressed as Gallic acid equivalents (GAE) in mg GAE per gram of dry weight (gdw) material.

Total Flavonoid Content (TFC)

Method: Chang et al. (2002)

Steps

1. Mix 0.5 mL of the extract with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water.
2. Incubate the mixture at room temperature for 30 minutes.
3. Measure the absorbance at 415 nm.

TFC is expressed as quercetin equivalent (mg/gdw).[20]

Lowry method

The Lowry assay, a widely used method for protein quantification, is a more sensitive variant of the Biuret assay, thanks to the use of Folin-phenol reagent that enhances colour development. This increased sensitivity allows the Lowry method to detect lower concentrations of protein than the Biuret assay, making it suitable for a variety of samples, including membrane proteins. The protocol for this method involves several steps, starting with the preparation of reagents and protein standards, followed by a series of reactions that result in a colour change measurable by a spectrophotometer.[11]

The procedure starts by preparing a stock solution of a standard protein, such as bovine serum albumin (BSA), at a concentration of 2 mg/mL in distilled water, stored frozen at −20°C. From this stock, serial dilutions are made to create standards ranging from 0 to 2000 µg/mL of protein.[10]

Reagents and Materials

The lowry assay uses three primary reagents

1. **Reagent A:** A solution of 100 g Na_2CO_3 in 1 litre of 0.5 N NaOH.
2. **Reagent B:** A 1% solution of copper sulphate ($CuSO_4 \cdot 5H_2O$) in distilled water.
3. **Reagent C:** A 2% solution of potassium tartrate in distilled water.

These reagents are stable and can be stored indefinitely. Before beginning the assay, these reagents are combined to form the working Lowry reagent (15 mL of Reagent A, 0.75 mL each of Reagents B and C). Additional materials include disposable cuvettes, test tubes, a 0.3 mg/mL BSA solution, 2N Folin-phenol reagent, and a vortex mixer. BSA serves as the standard.

Assay procedure

1. **Protein standards preparation:** In 10 separate test tubes, varying volumes (0 to 1.0 mL) of a 0.3 mg/mL BSA solution are pipetted, and each volume is brought to 1 mL with distilled water. This step creates a set of standards with different protein concentrations.

2. **Sample preparation:** Three other test tubes contain 0.1 mL of unknown protein samples, each also brought to 1 mL total volume with distilled water.

3. **Addition of lowry reagent:** To each tube, 1 mL of freshly prepared Lowry reagent is added. The tubes are vortex-mixed to ensure proper mixing and allowed to incubate at room temperature for 15 minutes.

4. **Folin-Phenol reagent addition:** During the incubation, a diluted Folin-phenol reagent is prepared by mixing 5 mL of 2N Folin-phenol reagent with 50 mL distilled water. After incubation, 3 mL of this diluted Folin-phenol reagent is quickly added to each tube, and the solutions are vortex-mixed. It is critical to complete this step quickly for each sample to prevent variability due to the unstable nature of the Folin reagent in alkaline conditions.

5. **Incubation:** The samples are incubated at room temperature for 45 minutes, during which the colour of the protein-dye complex develops.

6. **Absorbance Measurement:** The absorbance of each sample is measured at 540 nm using a spectrophotometer. For increased sensitivity, absorbance can also be measured at 750 nm.

7. **Calculation of Protein Concentration:** Using the absorbance values obtained from the known BSA concentrations, a standard curve is plotted. The absorbance values of the unknown samples are then compared to this curve to determine their protein concentrations.[11]

Important notes

1. **Sample preparation:** Samples available as precipitates should be dissolved in 2 N NaOH and hydrolysed as described. Whole cells or other complex samples may require pre-treatment to remove interfering substances. For example, trichloroacetic acid (TCA) or

perchloroacetic acid (PCA) can be used to precipitate proteins, separating them from DNA or other compounds.

2. **Interference and Sensitivity:** Several substances, including detergents, buffers, and nucleic acids, can interfere with the Lowry assay. Using SDS and a precipitation step, as described by Peterson, can eliminate these interferences. It is also important to maintain the pH between 10 and 10.5, as the reaction is highly pH-dependent. Adjustments to the Folin reagent addition can increase the assay's sensitivity.

3. **Variability:** The colour yield in the Lowry assay depends on the amino acid composition of the proteins. Different proteins at the same concentration can produce different absorbance values. Therefore, when using a standard curve based on BSA, the results are approximate unless the protein being tested is used to generate the standard curve.

4. **The lowry method:** while sensitive, has limitations due to interference and variability in colour yield among different proteins. With appropriate sample preparation and careful adherence to the protocol, the method remains a reliable technique for protein quantification.[10]

Synthesis of silver nanoparticles

Firstly, we prepared 500ml of silver nitrate solution of 0.001M, then by using magnetic stirrer, we stirred the silver nitrate solution at a rate of 600 rpm for about 10 mins then we slowly incorporated our extract in to the silver nitrate solution then allowed to stir it and then kept it undisturbed for 24 hours in a shady place then we subjected the solution to centrifugation. Where we centrifuged it at a rate of 10000 rpm. Then allowed he pellets to form, then we washed the pellets with deionized water thrice and then characterized the pellets using scanning electron microscopy analysis.[26]

Characterization

Scanning electron microscopy is a powerful tool for characterizing their morphology, size and surface features. The size of our silver nanoparticles were estimated by the scanning electron microscopy. Small particles with size of less than 100nm can be detected by scanning electron microscopy.[24]

An electron gun emits out a focused beam of high energy electrons that scans across the sample surface present in the sample chamber, when the electrons interact with the sample

they produce secondary electrons, detectors collect the secondary electrons and construct the high resolution image.[25]

Antimicrobial activity

The agar disc-diffusion method is a widely used microbiological technique to evaluate the antimicrobial activity of a substance. This method helps determine the effectiveness of antibiotics, disinfectants, and other antimicrobial agents against specific microorganisms.

In this procedure, a Petri dish containing a solidified agar medium is inoculated with a standardized suspension of the target microorganism. The inoculum is evenly spread across the surface using a sterile cotton swab to ensure uniform microbial growth. After inoculation, small filter paper discs (approximately 6 mm in diameter), impregnated with the antimicrobial agent at a predetermined concentration, were carefully placed on the agar surface. The antimicrobial agent gradually diffuses outward from the disc into the agar, creating a concentration gradient.

The inoculated plates are then incubated under appropriate temperature and atmospheric condition depending on the microbial strain being tested. Over time, the test microorganism begins to grow across the agar surface, except in areas where the antimicrobial agent is effective. If the compound exhibits antimicrobial properties, it inhibits microbial growth in the surrounding area, leading to the formation of a clear inhibition zone around the disc. The diameter of this inhibition zone is measured in millimeters using a ruler or caliper.

The size of the inhibition zone depends on several factors, including the diffusion rate of the antimicrobial agent, its potency, the susceptibility of the microorganism, and the agar composition. Larger inhibition zones typically indicate higher antimicrobial activity, whereas smaller zones suggest limited effectiveness.

This method is simple, cost-effective, and widely used in clinical microbiology, pharmaceutical research, and food safety testing. However, it provides qualitative rather than quantitative results, making it more suitable for preliminary screening rather than precise antimicrobial quantification.[23]

During our experiment we used this agar well diffusion method, where we also used a standard for the determination of antimicrobial activity. Firstly we sterilized the Petri dishes in the autoclave for 1.30 hours then we poured our agar medium in the petridishes. We took

3.8 grams of mueller hinton agar and mixed in 100 ml of water and incubated for 1 hour and then we took two microbes E coli and Bacillus after that and streaked them in Petri dishes and inoculated the microbes in it then we divided the petri dish by marking a line in between them and then incubated it for 24 hours and then measured the inhibition zone using a scale to determine its antimicrobial activity.

RESULT AND DISCUSSION

Growth of mung bean

1st Day 3rd Day 4th Day

5th Day 10th Day

Fig. 3: Growth stages of vigna radiata microgreens from germination to maturity.

Growth of red cow pea

1st Day 3rd Day 4th Day

6th Day **10th Day**

Fig. 4: Growth stages of vigna unguiculata microgreens.

Qualitative estimation of phytoconstituents

Table 1: Phytochemical analysis of vigna Radiate and Vigna unguiculata microgreens extract.

S. No.	Phytochemical constituents	Observation
1.	Carbohydrates	+
2.	Alkaloids	-
3.	Flavonoids	+
4.	Saponins	+
5.	Steroids	-
6.	Anthraquinone glycosides	-
7.	Cardiac glycosides	-
8.	Terpenoids	-
9.	Gum and Mucilages:	-
10.	Proteins	+

Quantitative estimation of phytoconstituents

Report

o The total flavonoid content of our extract is estimated as 69.9 ± 6.4 mg /g.

o The total phenolic content of our extract is estimated as 163.0 ± 11.1 mg/g**.**

Estimation of Protein by Lowry's method

Result: The given unknown sample contains 5.545µg protein/ml.

OBSERVATIONS AND CALCULATIONS

Table 3: Protein estimation using the lowry method.

Volume of standard BSA (ml)	Volume of distilled water (ml)	Concentration of Protein (µg)	Volume of Reagent C (ml)	Incubate At Room Temp for 10 min	Volume of reagent D (ml)	Incubate At dark room temp. for 30 min	Absorbance at 660
0.0	1.0	00	5		0.5		0.00
0.2	0.8	40	5		0.5		0.048
0.4	0.6	80	5		0.5		0.059
0.6	0.4	120	5		0.5		0.066
0.8	0.2	160	5		0.5		0.092
1.0	0.0	200	5		0.5		0.105
1.0 (sample)	0.0	5.5	5		0.5		0.097

Silver nanoparticle extract

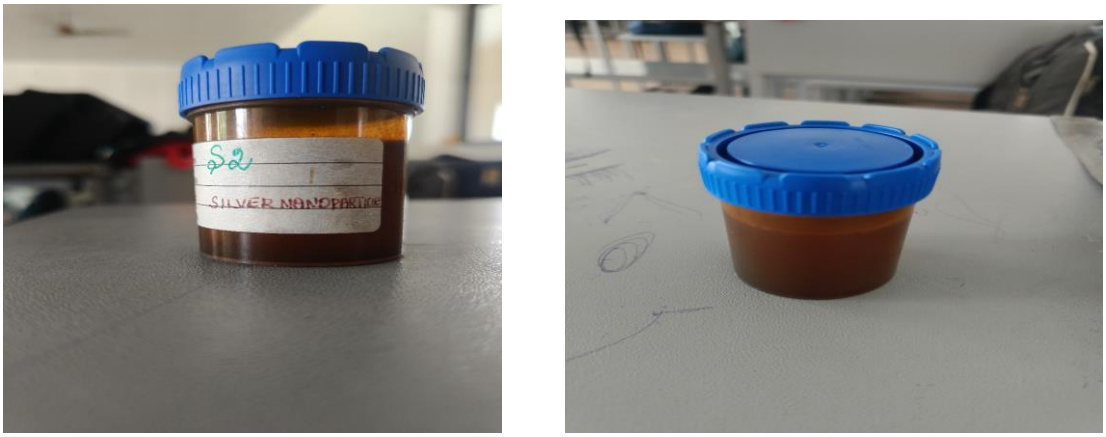


Fig. 5: Silver nanoparticle solution after synthesis from microgreens extract.

Scanning electron microscopy report

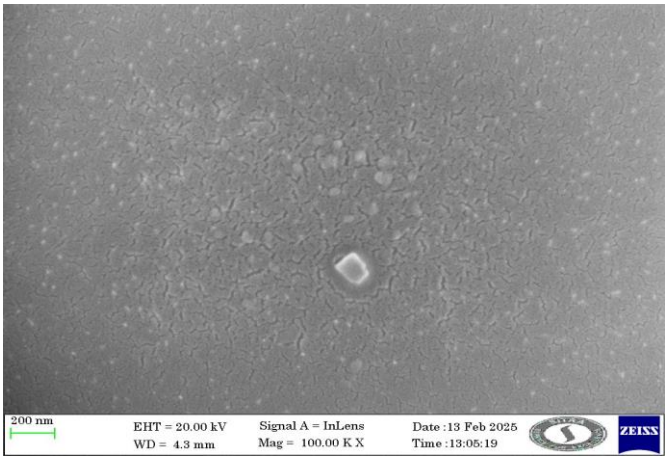
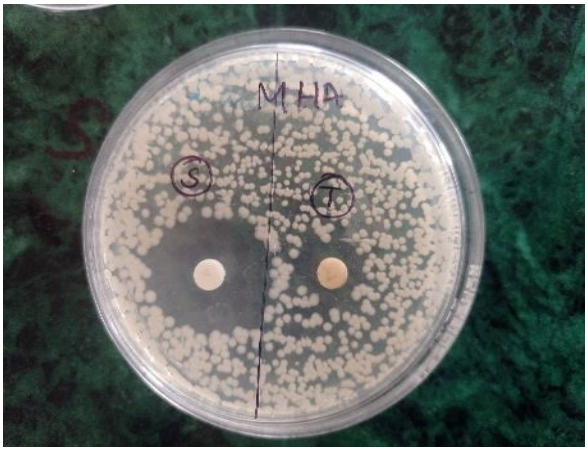


Fig. 6: Scanning electron microscopy image showing the morphology of synthesized silver nanoparticles.

Antimicrobial activity

Bacillus



Zone of Inhibition = 2cm

Fig. 7: Antimicrobial activity of silver nanoparticles against Bacillus sp. using agar well diffusion method.

E. Coli

Zone of Inhibition = 1cm

Fig. 8: Antimicrobial activity of silver nanoparticles against Escherichia coli using agar well diffusion method.

Comparision of antimicrobial activity

Table 4: Comparision of antimicrobial activity.

S. No	Microbes	Concentration	Zone of inhibition (standard)	Zone of inhibition (extract)
1.	Bacillus	Standard - 10µg/ml	4.3cm	2.5cm
2.	Escherichia coli	Sample – 12 mg/ml	3.6cm	2cm

DISCUSSION

Utilizing green and red peas for nanoparticle synthesis not only offers a safer and cost-effective approach but also aligns with the principles of green chemistry by minimizing environmental impact. The antioxidant properties inherent in these legumes can enhance the functionality of the synthesized nanoparticles, potentially increasing their efficacy in various applications. Overall, employing plant extracts for the synthesis of silver nanoparticles represents a significant advancement in nanotechnology, emphasizing the importance of exploring natural resources for sustainable practices in material science.[1]

Researchers have published an article indicating that the germination of mung bean sprouts significantly enhances their nutritional profile, particularly in terms of vitamin C content, total phenolic, total flavonoids, and total antioxidant activity compared to ungerminated mung bean seeds. This increase in bioactive compounds during the germination process highlights the potential health benefits of consuming mung bean sprouts. The elevated levels of phenolic and flavonoids, known for their antioxidant properties, suggest that these sprouts can play a vital role in combating oxidative stress and may contribute to overall health improvement.

Furthermore, the research emphasizes the importance of incorporating mung bean sprouts into a balanced diet. These sprouts are not only rich in essential nutrients but also serve as a potent source of bioactive compounds that can support immune function and reduce the risk of chronic diseases. The findings encourage consumers to enhance their dietary intake of fruits and vegetables, particularly those that are germinated, to achieve optimum nutrition. By doing so, individuals may better protect themselves against various health conditions, including cardiovascular diseases, diabetes, and certain cancers. Overall, this research underscores the nutritional advantages of mung bean sprouts and their potential role in preventive health strategies.[16]

Phenolic compounds and flavonoids are essential phytochemicals found in various plant species, including *Vigna radiata* (mung bean) and *Vigna unguiculata* (cowpea). These compounds are well-known for their potent antioxidant properties, which play a crucial role in protecting plant tissues from oxidative damage caused by environmental stressors. In addition to their protective functions in plants, phenolic and flavonoids offer numerous health benefits when consumed, including anti-inflammatory, antimicrobial, and anticancer activities.

The extraction method chosen for isolating the compounds which significantly influences the yield and composition of the phytochemicals obtained. Different extraction techniques, such as solvent extraction, maceration, and ultrasonic extraction, can affect the solubility and stability of phenolic and flavonoid compounds. In our experiment, we will carefully select and optimize the extraction process to ensure we effectively isolate and quantify the phenolic compounds and flavonoids from both *Vigna radiata* and *Vigna unguiculata.* By doing so, we aim to enhance our understanding of their phytochemical profiles and potential pharmacological activities. This knowledge will contribute to the growing body of research focused on the health benefits of these legumes and their role in promoting human health through functional foods and dietary supplements.[23]

CONCLUSION

The microgreens (*Vigna radiate and Vigna unguiculata)* grown and collected were extracted by using ethanol as a solvent. From the report of qualitative and quantitative estimation of phytoconstituents present in the combined extract, we concluded that the content of protein may serve as a good nutrient support for our human lives. Thus we proceed this work further into the formulation of Silver Nanoparticles by using the combined extract of microgreens and also determine antimicrobial activity.

We continue to do Formulation in future with the knowledge gained above.

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