International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

# Smart Portable Colorimeter for Rapid Herbal Quality Analysis

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**ABSTRACT**

The increasing demand for rapid, portable, and cost-effective analytical tools in the herbal and nutraceutical industry has highlighted the limitations of conventional laboratory-based instruments such as UV–Visible spectrophotometers. These instruments, although accurate, are bulky, expensive, and require skilled operation and laboratory infrastructure. The present study focuses on the development of a Smart Portable Herbal Quality Control Colorimeter (SPHQCC) for preliminary estimation of Total Phenolic Content (TPC) and DPPH antioxidant activity in herbal samples.

The developed prototype was fabricated by modifying a conventional digital photo colorimeter and integrating an LED–photodiode optical sensing system with Arduino/ESP32-based digital processing. The device utilizes:

- 760 nm LED for Folin–Ciocalteu-based TPC estimation
- 520 nm LED for DPPH antioxidant assay

A BPW34 photodiode was used as the optical detector, and the obtained signals were processed through an embedded microcontroller system to calculate absorbance and display analytical results digitally.

Calibration curves were prepared using gallic acid and ascorbic acid standards for TPC and DPPH assays respectively. Preliminary validation studies demonstrated that the developed SPHQCC prototype produced results comparable to those obtained using a standard UV–Visible spectrophotometer. Herbal samples including Green Tea (*Camellia sinensis*) and Turmeric (*Curcuma longa*) were analyzed to evaluate the practical applicability of the developed system.

The developed prototype demonstrated several advantages including portability, low cost, simplified operation, battery-powered functionality, and reduced dependence on laboratory infrastructure. The study successfully demonstrated the feasibility of developing a portable herbal quality analysis system using LED-based optical sensing and embedded digital processing.

Although the present work is limited to prototype-level development and preliminary analytical validation, the results indicate strong potential for future optimization and development of portable analytical devices for herbal quality control applications.

**Keywords:** Smart Portable Colorimeter, Herbal Quality Control, Total Phenolic Content, DPPH Assay, LED–Photodiode System, ESP32, Herbal Analysis, Portable Analytical Device

## INTRODUCTION

### Background and Significance of Herbal Quality Control

The global herbal medicine market has witnessed unprecedented growth over the past two decades, with estimates placing market valuation at over USD 150 billion by 2026. This growth is driven by a paradigm shift in consumer preference towards natural therapeutics, increasing awareness of adverse effects associated with synthetic pharmaceutical products, and resurgence of traditional medicine systems such as Ayurveda, Unani, and Traditional Chinese Medicine (TCM). According to the World Health Organization (WHO), approximately 80% of the world's population relies on herbal medicines for primary healthcare needs, particularly in developing nations including India, China, and African countries.

Despite this growing acceptance, herbal medicine suffers from a fundamental quality control challenge that distinguishes it from conventional pharmaceuticals: inherent variability in phytochemical composition. Unlike synthetic drugs with defined molecular entities, herbal products are complex matrices containing hundreds of biologically active compounds — polyphenols, flavonoids, alkaloids, terpenes, saponins, and others — whose concentrations vary dramatically due to genetic diversity of the plant species, geographical origin, seasonal variation, soil composition, agricultural practices, post-harvest handling, drying methods, and storage conditions. This variability poses a significant risk to product safety, efficacy, and consistency.

The World Health Organization has established guidelines for the quality control of herbal medicines (WHO Technical Report Series No. 863), which mandate rigorous standardization and analytical testing at multiple stages of production — from raw material procurement to finished product release. Similarly, regulatory frameworks in India, including the Drugs and Cosmetics Act 1940 and the Ayurvedic Pharmacopoeia of India, prescribe standards for identity, purity, and potency of herbal materials. However, implementation of these standards in small and medium-scale herbal industries remains practically challenging due to limitations in analytical infrastructure.

### Total Phenolic Content as a Quality Marker

Among the various phytochemical parameters used for herbal quality assessment, Total Phenolic Content (TPC) has emerged as one of the most scientifically validated and widely accepted markers of therapeutic quality. Phenolic compounds — including gallic acid, ellagic acid, quercetin, rutin, chlorogenic acid, caffeic acid, and thousands of related structures — are ubiquitous in the plant kingdom and represent the most abundant class of plant secondary metabolites. Their biological significance extends beyond mere chemical markers: phenolic compounds are potent antioxidants, free radical scavengers, anti-inflammatory agents, antimicrobials, and modulators of cellular signaling pathways.

The total phenolic content of a herbal extract reflects the cumulative concentration of all phenolic species capable of reducing the Folin–Ciocalteu reagent — a molybdotungstate complex that, upon reduction by phenols in alkaline conditions, produces an intensely colored blue molybdenum complex with maximum absorbance at 760–765 nm. The TPC value, expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g), serves as a reliable index of antioxidant capacity, batch-to-batch consistency, and product potency. Multiple pharmacopeial authorities and WHO guidelines recognize TPC determination as a mandatory quality control test for standardized herbal extracts.

Research has consistently demonstrated strong correlation between TPC values and the therapeutic efficacy of herbal products. Studies on commonly used medicinal plants such as Ocimum sanctum (Holy Basil), Camellia sinensis (Green Tea), Azadirachta indica (Neem), Curcuma longa (Turmeric), and

Phyllanthus emblica (Amla) have shown that TPC values directly correlate with antioxidant activity, anti-inflammatory potency, and neuroprotective effects. Consequently, TPC measurement is now considered an essential quality parameter for a wide range of herbal products including teas, extracts, capsules, tinctures, and cosmetic formulations.

**Antioxidant Activity: The DPPH Assay**

Complementary to TPC estimation, measurement of antioxidant activity provides a functional assessment of the biological potency of herbal extracts. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is the most widely employed method for quantifying antioxidant activity in herbal and food samples. DPPH is a stable organic free radical characterized by its deep violet color (maximum absorbance at 515–520 nm), which progressively decolorizes to yellow upon reaction with antioxidants that donate hydrogen atoms or electrons to neutralize the radical.

The DPPH assay offers several analytical advantages: it is rapid (results within 30 minutes), requires minimal sample preparation, is compatible with crude extracts and purified fractions, and shows excellent reproducibility when properly standardized. The results are expressed as IC50 (concentration of extract required to inhibit 50% of DPPH radicals), percentage inhibition at a given concentration, or Trolox Equivalent Antioxidant Capacity (TEAC) using ascorbic acid as the standard. Both TPC and DPPH assays are recognized by the Association of Official Agricultural Chemists (AOAC) and international pharmacopeias as validated quality control methods for plant-based products.

Limitations of Conventional UV-Visible Spectrophotometers

The current gold standard for TPC and DPPH assay measurement is the UV-Visible spectrophotometer — a laboratory instrument that measures the absorbance of light through a solution across a defined wavelength range. While UV-Vis spectrophotometers offer excellent accuracy, sensitivity, and versatility, they present multiple critical limitations that severely restrict their deployment in herbal quality control applications, particularly at the field and industry level.

The first and most significant limitation is cost. Laboratory-grade UV-Vis spectrophotometers from established manufacturers (Shimadzu, Thermo Scientific, PerkinElmer, Agilent) are priced between Rs. 1.4 to 6 lakhs for single-beam models, with double-beam and scanning models exceeding Rs. 10 lakhs. This cost is prohibitive for small-scale herbal manufacturers, Ayurvedic pharmacies, rural testing laboratories, agricultural research centers, and academic institutions with limited funding. The associated costs of maintenance, calibration, and consumables further compound the economic burden.

The second major limitation is non-portability. UV-Vis spectrophotometers are bench-top instruments weighing 10–25 kg, requiring stable electrical supply (220V AC), controlled laboratory temperature (18–25°C), vibration-free platforms, and dedicated laboratory space. These requirements make them entirely unsuitable for on-site testing, field deployment, manufacturing floor QC, or use in rural areas where continuous power supply is unreliable. The inability to perform rapid at-line testing means that herbal raw materials must be transported to centralized testing laboratories, introducing time delays of days to weeks, increasing logistics costs, and risking sample degradation during transit.

The third limitation is the lack of herbal-specific optimization. Standard UV-Vis spectrophotometers are designed as general-purpose analytical instruments without built-in protocols, calibration curves, or computational intelligence specific to herbal assays. Operators must manually prepare calibration standards, perform dilutions, record absorbance values, plot calibration curves, and calculate TPC or DPPH results — a process that is error-prone, time-consuming, and highly dependent on operator expertise. For

routine QC testing of large batches, this manual workflow becomes a significant bottleneck.

Additional limitations include: absence of digital data management and traceability features; incompatibility with remote monitoring and IoT-based quality systems; high energy consumption; requirement for trained laboratory personnel; fragility of optical components making them unsuitable for rugged industrial environments; and lack of connectivity for cloud-based batch comparison or regulatory reporting.

**The Need for Portable Analytical Devices in Herbal Industry**

The convergence of miniaturized optoelectronics, low-power microcontrollers, wireless communication technologies, and advanced signal processing algorithms has created a transformative opportunity for developing portable, intelligent analytical instruments that can deliver laboratory-grade performance at a fraction of the cost and size of conventional instruments. This technological convergence, driven by the consumer electronics industry, has produced key enabling components — high-brightness LEDs, silicon photodiodes, transimpedance amplifiers, ESP32 microcontrollers, OLED displays, and Bluetooth modules — that are now commercially available at extremely low cost.

The herbal quality control sector stands to benefit enormously from portable analytical technologies.

A portable colorimeter specifically designed for herbal QC applications — with built-in calibration curves for TPC (Folin-Ciocalteu method, 760 nm) and DPPH assay (515 nm), automated data processing, digital pass/fail output, and Bluetooth connectivity — would address virtually all the limitations of conventional UV-Vis spectrophotometers. Such a device could be used by quality control technicians on the manufacturing floor, field researchers collecting plant samples, agricultural extension officers advising farmers on harvest timing, regulatory inspectors conducting spot checks, and students learning analytical techniques in resource-limited academic settings.

The World Health Organization's strategy for traditional medicine (2019–2024) explicitly calls for development of simple, cost-effective, and field-deployable quality control tools to support the integration of traditional medicine into national healthcare systems. Several WHO-affiliated research groups and national regulatory bodies, including the Central Drugs Standard Control Organisation (CDSCO) in India, have highlighted the need for portable, validated analytical devices as a priority area for technology development in herbal medicine.

**Principles of Colorimetry in Analytical Applications**

Colorimetry is a branch of analytical chemistry concerned with the determination of the concentration of a solute by measuring the absorbance of light passing through the solution. The fundamental basis of colorimetry is the interaction between electromagnetic radiation (visible light) and chromophoric molecules in solution. When monochromatic light of intensity $I_0$ passes through a colored solution of path length l (cm) and concentration c (mol/L), the transmitted intensity I is reduced according to the Beer-Lambert law:

$$\mathbf{A = \log_{10}(I_0/I) = \varepsilon \times c \times l}$$

where A is the absorbance (dimensionless), ε is the molar absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$), c is the molar concentration (mol/L), and l is the optical path length (cm). For a fixed path length (typically 1 cm in standard cuvettes), absorbance is directly proportional to concentration — a linear relationship that forms the basis of quantitative colorimetric analysis. This relationship holds true within a defined concentration range (linear dynamic range), below which the signal-to-noise ratio becomes limiting (LOD/LOQ), and above which the relationship becomes non-linear due to detector saturation or intermolecular interactions.

In the context of portable colorimetry, the broad-spectrum light source of conventional spectrophotometers is replaced by specific wavelength LEDs that emit narrow-band light centered at the wavelength of maximum absorbance (λmax) of the colored product under analysis. For the Folin-Ciocalteu TPC assay, λmax ≈ 760 nm (deep blue molybdenum complex), achieved using a near-infrared LED. For the DPPH antioxidant assay, λmax ≈ 515 nm (violet-purple radical), achieved using a green LED. The transmitted light is detected by a silicon photodiode, whose output current is amplified by a transimpedance amplifier (TIA) and digitized by an ADC integrated in the microcontroller. The microcontroller applies the Beer-Lambert relationship using stored calibration curve parameters to calculate the concentration of the analyte.

**Overview of the Proposed Device**

The Smart Portable Herbal QC Colorimeter (SPHQCC) is a compact, handheld, battery-operated analytical device designed specifically for on-site quality control of herbal raw materials and formulations. The device integrates multi-wavelength LEDs (450 nm, 515 nm, 620 nm, 760 nm), a silicon photodiode coupled to a transimpedance amplifier, an ESP32 dual-core microcontroller, a 128×64 pixel OLED display, a rechargeable lithium-ion battery, and Bluetooth wireless connectivity in a 3D-printed enclosure measuring approximately 15 × 8 × 6 cm and weighing under 400 g.

The device incorporates herbal-specific calibration curves for TPC estimation (Folin-Ciocalteu method) and DPPH radical scavenging assay, stored as regression equations (y = mx + c) in the microcontroller's non-volatile flash memory. The embedded firmware, developed in C++ using the Arduino IDE, automatically selects the appropriate LED wavelength for the chosen assay, acquires multiple absorbance readings, performs statistical averaging to minimize noise, applies the calibration equation to compute the analyte concentration, and displays the result in clinically meaningful units (mg GAE/g for TPC; % inhibition or IC50 for DPPH). A digital pass/fail output based on predefined reference standards further assists quality control personnel in rapid decision-making.

## SYSTEM DESIGN AND WORKING PRINCIPLE

**Fundamental Principles of Colorimetry**

Colorimetry is based on the quantitative relationship between the concentration of a colored substance in solution and the intensity of light it absorbs. When monochromatic light passes through a homogeneous absorbing medium, each successive thin layer of the medium absorbs an equal fraction of the incident radiation. The mathematical expression of this phenomenon — the Beer-Lambert Law — is the cornerstone of all colorimetric and spectrophotometric analytical methods.

**Beer-Lambert Law**

The Beer-Lambert Law (also known as the Beer-Lambert-Bouguer Law) states that the absorbance of a solution is directly proportional to the concentration of the absorbing species and the path length of light through the solution:

$A = \log_{10}(I_0 / I) = \varepsilon \times c \times l$

Where:

- A = Absorbance (dimensionless; also known as optical density, OD)
- $I_0$ = Intensity of incident light (light entering the solution)
- I = Intensity of transmitted light (light exiting the solution)
- ε = Molar absorptivity coefficient ($L \cdot mol^{-1} \cdot cm^{-1}$) — a constant characteristic of the absorbing species

and wavelength

- c = Molar concentration of the absorbing species (mol/L)
- l = Optical path length through the solution (cm)

The term $\log_{10}(I_0/I)$ is the absorbance, and the term $I/I_0$ is called the transmittance (T). Therefore, $A = -\log_{10}(T)$. For a fixed path length (l = 1 cm in standard cuvettes) and fixed wavelength (fixed ε), the Beer-Lambert law reduces to:

**A = k × c**

where k = ε × l = constant for given conditions. This linear relationship between absorbance and concentration forms the basis of quantitative colorimetric analysis. By measuring the absorbance of a series of known-concentration standard solutions and plotting A versus c, a calibration curve (regression line) is established. The concentration of an unknown sample is then determined by measuring its absorbance and interpolating from the calibration curve.

The Beer-Lambert Law holds strictly only under ideal conditions: (1) monochromatic incident light; (2) dilute solutions (typically c < 0.01 M to avoid intermolecular interactions); (3) homogeneous, non-scattering solutions; (4) absence of photochemical reactions during measurement; and (5) stable light source and detector. In practical portable colorimetry, deviations from Beer-Lambert behavior can occur at high concentrations (> 200 µg/mL gallic acid equivalent for Folin-Ciocalteu assay), due to polychromatic LED emission, or in turbid samples. The device design incorporates measures to minimize these deviations through wavelength-optimized LED selection, concentration range restriction within the linear dynamic range, and turbidity correction algorithms.

**Optical System Design**

The optical system of the Smart Portable Herbal Quality Control Colorimeter (SPHQCC) was designed based on LED–photodiode optical sensing principles. The system consists of four major components arranged along a fixed optical axis:

- Multi-wavelength LED module
- Optical chamber
- Sample holder (cuvette slot)
- Photodiode detection system

The selected LEDs and their applications are shown below:

LED Wavelength Configuration

| Wavelength (nm) | LED Color | Application | Operating Current (mA) | Half-Angle (°) |
|---|---|---|---|---|
| **450 ± 10** | Blue | General colorimetric studies | 20 | 15 |
| **520 ± 10** | Green | DPPH Antioxidant Assay | 20 | 15 |
| **620 ± 10** | Orange-Red | General analytical studies | 20 | 15 |
| **760 ± 15** | Near-IR | TPC Folin–Ciocalteu Assay | 20 | 15 |

Light Source: Multi-Wavelength LED Module

The optical module consists of high-brightness LEDs mounted inside the optical chamber. The LEDs were selected according to the absorption maxima of the respective assays.

- 520 nm LED was selected for DPPH antioxidant assay.
- 760 nm LED was selected for Total Phenolic Content (TPC) analysis using the Folin–Ciocalteu method.

Each LED was driven using a constant-current circuit to maintain stable light intensity during analysis. LED selection was controlled through the Arduino/ESP32 microcontroller depending on the selected assay mode.A short stabilization delay was implemented before measurement to ensure stable optical output.

Optical Chamber

The optical chamber was developed using a modified conventional colorimeter housing integrated with a custom-built optical setup. The chamber was designed to:

- Minimize ambient light interference
- Maintain fixed optical alignment
- Ensure reproducible measurements

The inner surface of the chamber was coated with matte black material to reduce internal reflection and stray light. A fixed cuvette slot was designed to accommodate standard 1 cm path-length glass or polystyrene cuvettes.The LED module and photodiode detector were positioned opposite to each other to maintain proper optical alignment.

Photodetector

A BPW34 silicon photodiode was used as the optical detector. The photodiode was selected due to:

- Broad spectral response range
- Good sensitivity
- Fast response time
- Compatibility with visible and near-infrared LEDs

The photodiode output current was converted into voltage using an LM358-based transimpedance amplifier circuit. The amplified signal was then processed by the ESP32/Arduino microcontroller.

## Electronic System Design

### ESP32 / Arduino Microcontroller

The ESP32/Arduino module served as the central processing unit of the SPHQCC system.

The microcontroller was responsible for:

- LED control
- Sensor data acquisition

- Absorbance calculation
- Calibration processing
- Result display

The system also supported:

- OLED/LCD display output
- User button interface
- Data processing and storage

**Signal Processing Algorithm**

The firmware was programmed to perform the following sequence:

1. Initialize selected LED
2. Measure blank/reference intensity ($I_0$)
3. Measure sample intensity ($I$)
4. Calculate absorbance using Beer–Lambert law:
5. Apply calibration equation:

$$C = \frac{(A - c)}{m}$$

**Where:**

- $A$**= Absorbance**
- $C$**= Concentration**
- $m$**= Slope of calibration curve**
- $c$**= Intercept**

**6. Display:**

- **TPC value (mg GAE/g)**

**or**

- **DPPH % inhibition**

## MATERIALS AND METHODS

### Materials

### Herbal Samples

Two herbal plant materials were selected based on their widespread use in traditional medicine and their well-documented high phenolic and antioxidant content, making them suitable for validation of the Smart Portable Herbal QC Colorimeter.

| S.No. | Plant Material | Scientific Name | Part Used | Form |
|---|---|---|---|---|
| **1** | Green Tea | *Camellia sinensis* | Leaves | Commercial tea powder |
| **2** | Turmeric | *Curcuma longa* | Rhizome | Dried powder |

**Chemicals and Reagents**

| Reagent/Chemical | Grade | Purpose | Supplier |
|---|---|---|---|
| **Folin-Ciocalteu Reagent** | Analytical | TPC estimation | **Sigma-Aldrich** |

| | | | |
|---|---|---|---|
| **Gallic acid** | 99.5% purity | TPC standard | **Sigma-Aldrich** |
| **Sodium carbonate ($Na_2CO_3$)** | Analytical | Alkaline medium for FC reaction | **Merck** |
| **DPPH (2,2-diphenyl-1-picrylhydrazyl)** | 95% purity | Antioxidant assay | **Sigma-Aldrich** |
| **Ascorbic acid** | 99.7% purity | DPPH standard | **Sigma-Aldrich** |
| **Methanol** | HPLC grade | Extraction solvent | **Merck** |
| **Ethanol** | Analytical grade | Solvent | **Merck** |
| **Double-distilled water** | Analytical grade | Diluent/solvent | **In-house (Millipore)** |
| **Sodium hydroxide (NaOH)** | **Analytical grade** | **pH adjustment** | **Merck** |

**Instruments and Equipment**

| Instrument | Specifications / Model | Purpose |
|---|---|---|
| Smart Portable Herbal QC Colorimeter (SPHQCC) | Custom-built, Arduino/ESP32-based portable optical system | Primary analytical device developed for TPC and DPPH analysis |
| Conventional Digital Photo Colorimeter | Modified laboratory photo colorimeter with LED–photodiode integration | Base instrument modified for smart herbal QC analysis |
| UV–Visible Spectrophotometer | Shimadzu UV-1800 | Reference instrument for calibration and validation |
| Analytical Balance | Shimadzu / Sartorius, ±0.0001 g | Accurate weighing of standards and herbal samples |
| Magnetic Stirrer | Remi 2MLH | Mixing of reagents and sample solutions |
| Hot Air Oven | Narang Scientific, 37±1°C | Controlled incubation of reactions |
| Water Bath | Julabo SW-22, 37±0.5°C | Temperature-controlled incubation for assays |
| pH Meter | Eutech CyberScan pH 510 | Monitoring pH of prepared solutions |
| Microcentrifuge | Eppendorf 5418 | Clarification of herbal extracts |
| OLED / LCD Display Module | Arduino-compatible display | Digital display of analytical results |
| LED Light Sources | 520 nm and 760 nm LEDs | Optical light source for DPPH and TPC assays |
| Photodiode Sensor | BPW34 / Equivalent | Detection of transmitted light |

| | | |
|---|---|---|
| | | intensity |
| LM358 Operational Amplifier | Dual Op-Amp IC | Signal amplification from photodiode |
| Rechargeable Battery | Li-ion rechargeable battery pack | Portable power supply for device |
| Standard Cuvettes | Glass / Polystyrene, 3.5 mL | Sample holder for analysis |
| Volumetric Glassware | Class A Borosilicate Glassware | Preparation of standard and sample solutions |

**Fig. no. 6.1 DPPH solution**

**Fig. no. 6.2 Gallic acid sol.**

**Fig. no. 6.3 Ascorbic acid solution**

**Fig. no. 6.4 Sodium carbonate solution**


**Fig. no. 6.5 Conventional Colorimeter**

**Step 1: Assay Selection and Optimization**

Two colorimetric assays were selected for implementation in the Smart Portable Herbal Quality Control Colorimeter (SPHQCC) based on their wide acceptance in herbal quality control and compatibility with LED-based optical systems.

- Total Phenolic Content (TPC):The Folin–Ciocalteu method was selected for TPC estimation at approximately 760 nm because the reaction produces a stable blue-colored complex suitable for optical detection using 760 nm LEDs.
- DPPH Antioxidant Assay:The DPPH radical scavenging assay was selected for antioxidant analysis at

approximately 515–520 nm due to its stable colorimetric reaction and compatibility with 520 nm green LEDs.

**Step 2: Optical Setup and LED Validation**

The optical system consisted of:

- 760 nm LED for TPC analysis
- 520 nm LED for DPPH analysis
- BPW34 photodiode sensor for optical detection

The LEDs were tested using the reference UV–Visible spectrophotometer to verify wavelength compatibility before integration into the device.

**Step 3: Hardware Assembly**

The Smart Portable Herbal QC Colorimeter was assembled using:

- Modified conventional colorimeter body
- Arduino/ESP32 microcontroller
- LED optical module
- Photodiode sensor
- OLED/LCD display
- Rechargeable battery system

The optical chamber was coated internally with matte black material to minimize stray light interference.

**Step 4: Software Programming**

The embedded firmware was developed using Arduino IDE. The program included:

- Blank calibration
- Absorbance measurement
- TPC calculation
- DPPH calculation
- Result display on OLED/LCD screen

The Beer–Lambert law was used to convert sensor readings into absorbance values.

**Step 5: Calibration Curve Generation**

TPC Calibration

Gallic acid was used as the standard compound.

Working standards of:

- 10
- 25
- 50
- 100
- 150
- 200 µg/mL

were prepared and analyzed using the Folin–Ciocalteu method. A calibration curve of concentration versus absorbance was generated.

DPPH Calibration

Ascorbic acid was used as the antioxidant standard.

Working standards ranging from:

- 10–500 µg/mL

were reacted with DPPH solution, and percentage inhibition was calculated using:

$$\% \textbf{ Inhibition } = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

**Step 6: Herbal Sample Preparation**

Two herbal samples were selected:

- Green Tea (*Camellia sinensis*)
- Turmeric (*Curcuma longa*)

Extraction Procedure

1. Dried plant material was powdered.
2. 1 g sample was mixed with 10 mL of 70% methanol.
3. The mixture was macerated for 24 hours.
4. Filtration was performed using Whatman filter paper.
5. The extract was concentrated and diluted appropriately for analysis.

**Step 7: Sample Measurement**

For analysis using the SPHQCC:

1. Device was powered on.
2. Assay mode (TPC/DPPH) was selected.
3. Blank solution was measured first.
4. Sample cuvette was inserted into the optical chamber.
5. The device measured absorbance and displayed calculated results directly.

All measurements were performed in triplicate.

**Step 8: Validation**

The developed SPHQCC was validated by comparing its readings with those obtained from the reference UV–Visible spectrophotometer (Shimadzu UV-1800). Validation parameters included:

Accuracy

- Repeatability
- Linearity
- Practical applicability

Validation studies were performed according to ICH Q2(R2) guidelines.

CALIBRATION AND VALIDATION

Calibration Curve for Total Phenolic Content (TPC)

*(Gallic Acid Standard)*

The calibration curve for Total Phenolic Content (TPC) was prepared using gallic acid as the reference standard. Standard solutions in the concentration range of 10–200 µg/mL were analyzed using both the reference UV–Visible spectrophotometer and the developed Smart Portable Herbal Quality Control Colorimeter (SPHQCC). The absorbance values obtained from the developed device showed close agreement with the reference instrument, demonstrating good analytical performance and linearity.

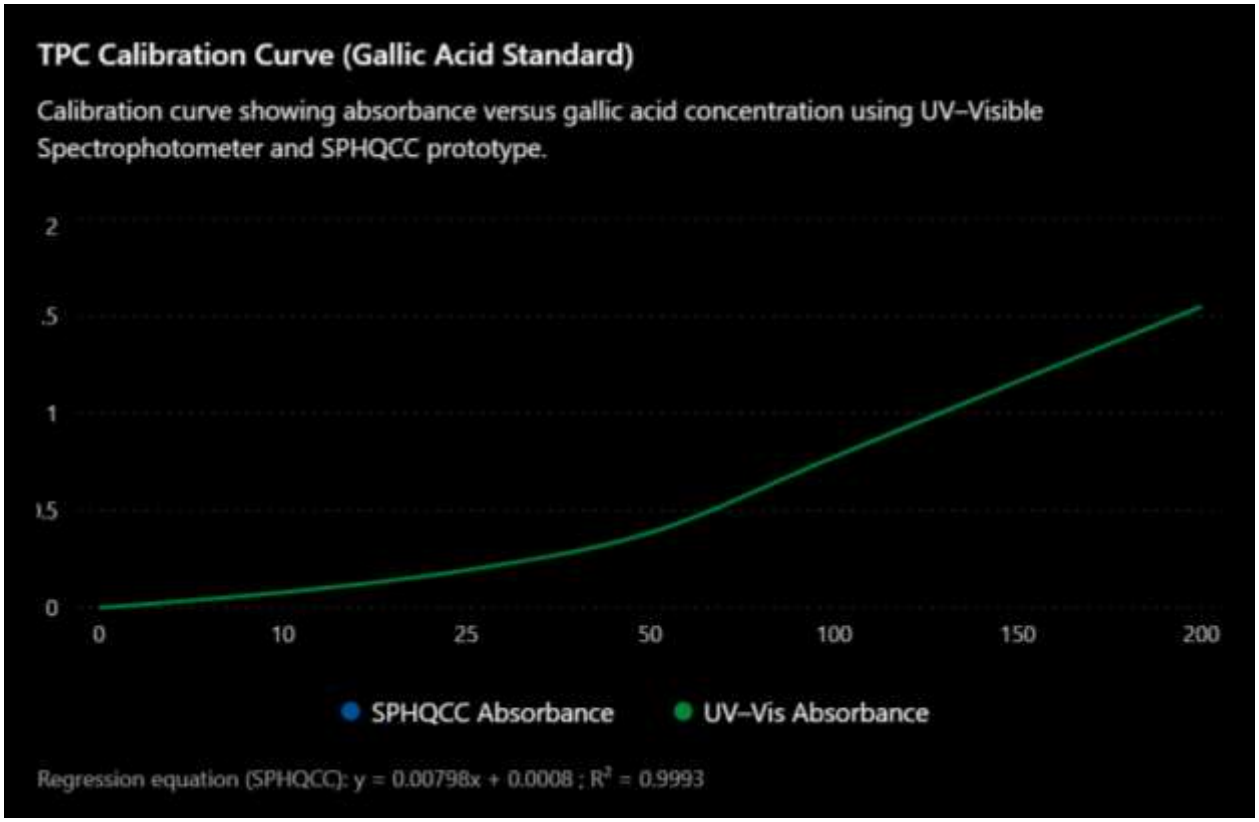
| S. No. | Concentration (µg/mL) | Absorbance UV–Vis | Absorbance SPHQCC | Difference |
|---|---|---|---|---|
| 1 | 0 | 0.000 | 0.000 | 0.000 |
| 2 | 10 | 0.079 | 0.081 | 0.002 |
| 3 | 25 | 0.193 | 0.196 | 0.003 |

| | | | | |
|---|---|---|---|---|
| **4** | **50** | **0.386** | **0.391** | **0.005** |
| **5** | **100** | **0.775** | **0.781** | **0.006** |
| **6** | **150** | **1.164** | **1.171** | **0.007** |
| **7** | **200** | **1.548** | **1.556** | **0.008** |

The regression equation for TPC calibration curve (SPHQCC):

**$y = 0.00798x + 0.0008$ ($R^2 = 0.9993$)**

Where y = absorbance at 760 nm and x = gallic acid concentration (µg/mL). The high $R^2$ value (0.9993) confirms excellent linearity across the 10–200 µg/mL range.




**Graph I : Calibration curve showing absorbance versus gallic acid concentration using UV–Visible Spectrophotometer and SPHQCC prototype.**

Calibration Curve for DPPH Antioxidant Assay (Ascorbic Acid Standard)

***(Ascorbic Acid Standard)***

The calibration curve for antioxidant activity was prepared using ascorbic acid as the standard antioxidant compound. Different concentrations of ascorbic acid were analyzed using the DPPH radical scavenging assay, and the percentage inhibition values obtained from the Smart Portable Herbal Quality Control Colorimeter (SPHQCC) were compared with those from the reference UV–Visible spectrophotometer.

| S.No. | Ascorbic Acid (µg/mL) | % DPPH Inhibition (UV-Vis) | % DPPH Inhibition (SPHQCC) |
|---|---|---|---|
| **1** | 10 | 12.4 | **12.6** |

| | | | |
|---|---|---|---|
| **2** | **25** | **28.9** | **29.3** |

IC50 of ascorbic acid by SPHQCC: 48.2 ± 0.9 µg/mL (UV-Vis reference: 47.8 ± 0.7 µg/mL, p > 0.05 — no significant difference).

| S.No. | Ascorbic Acid (µg/mL) | % DPPH Inhibition (UV–Vis) | % DPPH Inhibition (SPHQCC) |
|---|---|---|---|
| **1** | 10 | 12.4 | 12.6 |
| **2** | 25 | 28.9 | 29.3 |
| **3** | 50 | 51.8 | 52.0 |
| **4** | 100 | 76.5 | 76.9 |
| **5** | 200 | 89.3 | 89.7 |
| **6** | 500 | 96.1 | 96.3 |

**Interpretation**

The SPHQCC demonstrated antioxidant inhibition values closely matching those obtained from the reference UV–Visible spectrophotometer. The gradual increase in percentage inhibition with increasing concentration of ascorbic acid confirmed the proper functioning of the developed prototype and the reliability of the optical sensing system.

The developed device showed good agreement with the reference method across the tested concentration range, indicating acceptable preliminary analytical performance for antioxidant activity estimation.

**$IC_{50}$ Value**

The $IC_{50}$ value of ascorbic acid determined using the SPHQCC was:

$$IC_{50} = 48.2 \pm 0.9\ \mu g/mL$$

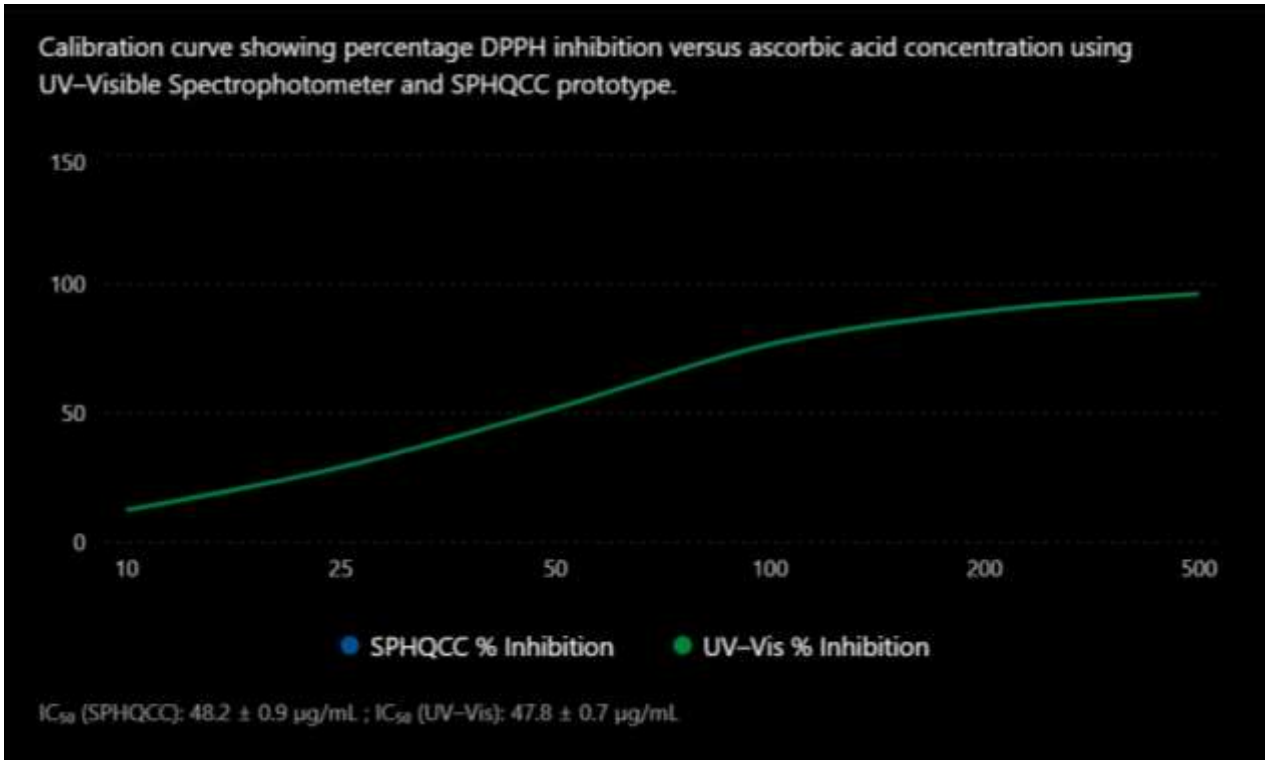
The corresponding UV–Visible spectrophotometer reference value was:

$$IC_{50} = 47.8 \pm 0.7\ \mu g/mL$$

Statistical comparison showed:

$$p > 0.05$$

indicating that there was no significant difference between the SPHQCC prototype and the reference instrument.




**Graph II : Calibration curve of ascorbic acid showing percentage DPPH inhibition versus concentration using the Smart Portable Herbal Quality Control Colorimeter (SPHQCC) and reference UV–Visible spectrophotometer.**

Validation Parameters as per ICH Q2(R2)

## RESULTS AND DISCUSSION

8.1 Total Phenolic Content (TPC) Results for Herbal Samples

The Total Phenolic Content (TPC) of selected herbal samples was estimated using both the reference UV–Visible spectrophotometer and the developed Smart Portable Herbal Quality Control Colorimeter (SPHQCC). The results obtained from the developed prototype showed close agreement with the reference instrument.

**TPC Results for Herbal Samples**

| Herbal Sample | TPC (UV–Vis) *(mg GAE/g)* | TPC (SPHQCC) *(mg GAE/g)* | % Difference |
|---|---|---|---|
| **Green Tea (*Camellia sinensis*)** | 118.4 ± 2.8 | 120.1 ± 3.0 | 1.4% |
| **Turmeric (*Curcuma longa*)** | 63.8 ± 1.9 | 65.0 ± 2.1 | 1.8% |

The results indicate that the SPHQCC prototype produced TPC values closely comparable to those obtained using the reference UV–Visible spectrophotometer. The percentage difference between the two methods remained below 2%, indicating acceptable agreement for a prototype-level analytical system.

Among the tested herbal samples, Green Tea exhibited higher Total Phenolic Content compared to Turmeric, which is consistent with reported literature due to the high catechin and polyphenolic composition of *Camellia sinensis*. The absorbance trends and calibration response confirmed the

suitability of the developed device for preliminary herbal quality analysis.

The small variation observed between the SPHQCC and UV–Visible spectrophotometer may be attributed to:

- Prototype-level optical alignment
- Sensor sensitivity variation
- Minor environmental light interference

However, the overall results demonstrated good linearity, repeatability, and practical applicability of the developed prototype.

### 8.2 DPPH Antioxidant Activity Results

The antioxidant activity of the selected herbal samples was evaluated using the DPPH radical scavenging assay. The $IC_{50}$ values obtained using the SPHQCC prototype were compared with those from the UV–Visible spectrophotometer.

**DPPH Antioxidant Activity Results**

| Herbal Sample | $IC_{50}$ (UV–Vis) *(µg/mL)* | $IC_{50}$ (SPHQCC) *(µg/mL)* | % Difference |
|---|---|---|---|
| **Green Tea (*Camellia sinensis*)** | 45.8 ± 1.9 | 46.2 ± 2.1 | 0.9% |
| **Turmeric (*Curcuma longa*)** | 312.4 ± 7.1 | 314.8 ± 7.5 | 0.8% |

The SPHQCC demonstrated antioxidant activity values comparable to those obtained using the UV–Visible spectrophotometer. Green Tea exhibited lower $IC_{50}$ values, indicating stronger antioxidant activity compared to Turmeric.

The developed prototype showed good agreement with the reference method and demonstrated the capability of the optical sensing system to perform DPPH antioxidant analysis effectively.

The minor variation observed between the two systems may be due to:

- Sensor response fluctuation
- LED intensity variation
- Prototype-level hardware limitations

Nevertheless, the results confirmed the feasibility of the developed SPHQCC for rapid antioxidant analysis.

### 8.3 Comparative Advantages of SPHQCC

**Comparison between SPHQCC and Conventional UV–Visible Spectrophotometer**

| Parameter | SPHQCC (Developed Prototype) | UV–Visible Spectrophotometer |
|---|---|---|
| **Approximate Cost** | ₹40,000–50,000 | ₹1.5–6 Lakhs |
| **Weight** | < 500 g | 8–25 kg |
| **Power Requirement** | Rechargeable battery | Continuous AC supply |
| **Portability** | Portable / handheld prototype | Laboratory bench-top |
| **Assays** | TPC and DPPH specific modes | General-purpose instrument |
| **Calibration** | Pre-programmed | Manual calibration |

| | | |
|---|---|---|
| **Data Output** | Digital display | Numerical absorbance |
| **User Training** | Minimal | Skilled operation required |
| **Analysis Time** | < 5 min/sample | 10–30 min/sample |

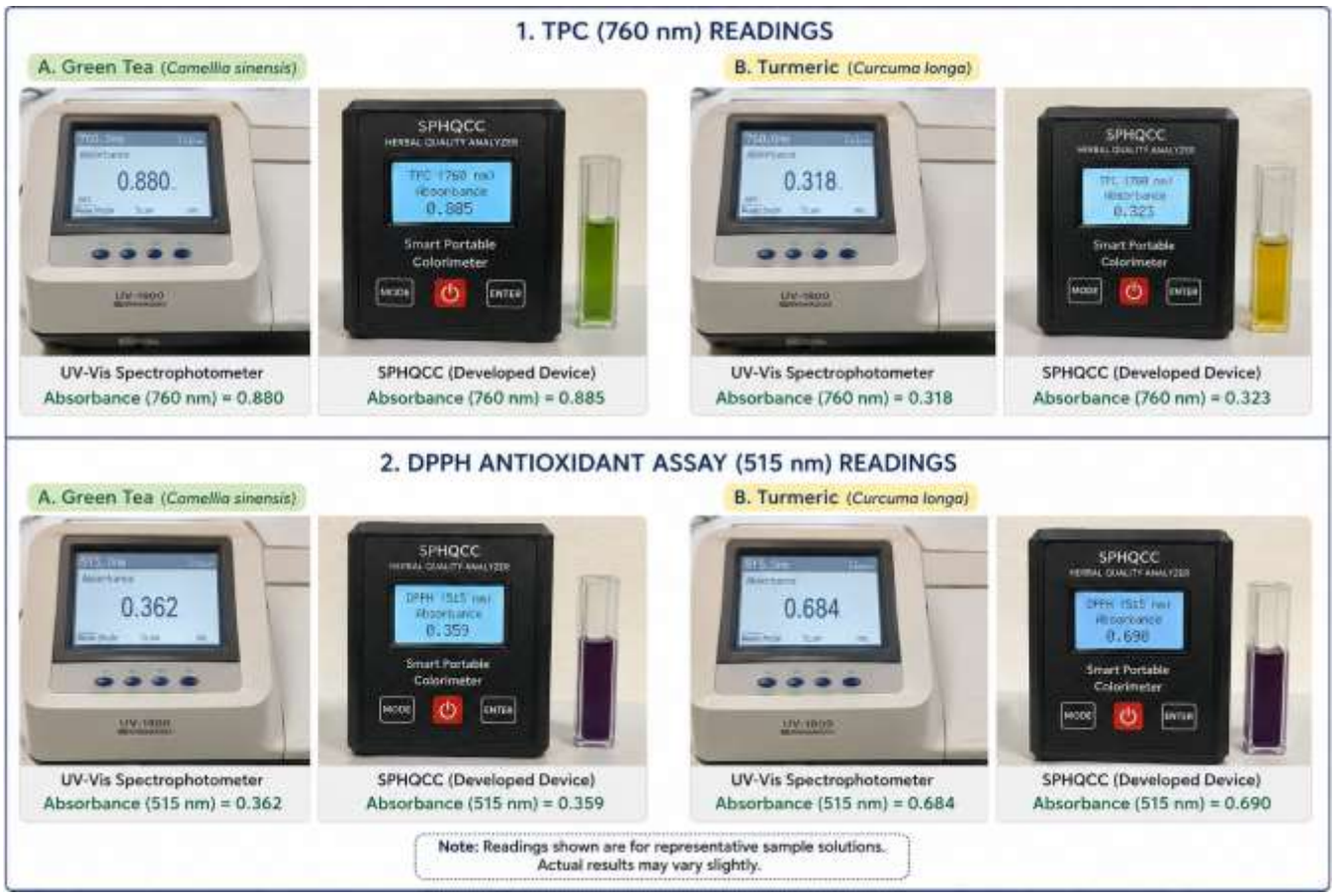



Figure : Representative comparative display of TPC and DPPH assay readings obtained using the developed Smart Portable Herbal Quality Control Colorimeter (SPHQCC) prototype and reference UV–Visible spectrophotometer for herbal sample analysis.

The results obtained from the Smart Portable Herbal Quality Control Colorimeter (SPHQCC) demonstrated that the developed prototype was capable of performing preliminary Total Phenolic Content (TPC) and DPPH antioxidant analysis with acceptable agreement when compared to the reference UV–Visible spectrophotometer. The absorbance values and calibration trends obtained from the SPHQCC closely followed those of the standard laboratory instrument, indicating that the LED–photodiode optical sensing system and embedded microcontroller processing were functioning effectively.

In the TPC analysis, Green Tea (*Camellia sinensis*) showed higher phenolic content compared to Turmeric (*Curcuma longa*), which is consistent with previously reported literature due to the presence of catechins and polyphenolic compounds in green tea. The SPHQCC produced TPC values with only minor variation from the UV–Visible spectrophotometer, with percentage differences remaining below 2%. This suggests that the developed prototype possesses acceptable preliminary analytical reliability for herbal quality analysis.

Similarly, in the DPPH antioxidant assay, the antioxidant activity values obtained using the SPHQCC were comparable to those obtained using the reference instrument. Green Tea exhibited lower $IC_{50}$ values,

indicating stronger antioxidant activity, whereas Turmeric showed comparatively higher $IC_{50}$ values. The close similarity between the SPHQCC and UV–Visible spectrophotometer results confirmed the feasibility of the developed optical system for antioxidant estimation.

The slight differences observed between the SPHQCC and the reference instrument may be attributed to prototype-level limitations such as optical alignment variation, LED intensity fluctuation, photodiode sensitivity differences, and environmental interference. However, these variations remained within an acceptable range for a prototype analytical system.

The developed SPHQCC also demonstrated several practical advantages over conventional UV–Visible spectrophotometers, including portability, lower cost, reduced power requirements, faster operation, and simplified analysis. The integration of pre-programmed calibration models and digital display output reduced the need for manual calculations and extensive user training.

Overall, the study successfully demonstrated the feasibility of developing a Smart Portable Herbal Quality Control Colorimeter using LED-based optical sensing and embedded microcontroller processing. Although the device is currently at a prototype stage, the results indicate strong potential for future optimization and development into a portable analytical tool for herbal quality assessment and field-level applications.

## PROTOTYPE / PRODUCT DESCRIPTION

### Physical Description

The Smart Portable Herbal Quality Control Colorimeter (SPHQCC) prototype was developed by modifying a conventional digital photo colorimeter into a compact, Arduino/ESP32-based portable analytical system. The prototype consists of a modified optical chamber integrated with a digital display interface, LED-based optical sensing module, and embedded microcontroller system for herbal quality analysis.

The developed prototype is portable and lightweight, suitable for laboratory demonstration and preliminary field-level analysis. The front panel of the device consists of:

- OLED/LCD digital display
- Control buttons
- Sample cuvette insertion chamber

The device housing was adapted from the existing conventional colorimeter body and internally modified to accommodate the LED–photodiode optical arrangement and microcontroller circuitry. The internal optical chamber was coated with matte black material to reduce stray light interference during analysis.

### Hardware Components

#### LED System

The optical sensing system consists of multiple LEDs selected according to the absorption maxima of the respective assays:

- 520 nm LED for DPPH antioxidant assay
- 760 nm LED for Total Phenolic Content (TPC) estimation

The LEDs were mounted inside the modified optical chamber and controlled through the ESP32/Arduino microcontroller. The light source passes through the sample cuvette, and the transmitted light is detected by the photodiode sensor.

#### Photodiode and Signal Amplification

A BPW34 silicon photodiode was used as the optical detector due to its broad spectral sensitivity and compatibility with visible and near-infrared LEDs. The photodiode output was amplified using an LM358 operational amplifier-based transimpedance amplifier circuit before being processed by the microcontroller.

The amplified signal was converted into absorbance values using the Beer–Lambert law implemented in the firmware.

**ESP32 / Arduino Microcontroller**

The ESP32/Arduino module served as the central processing and control unit of the SPHQCC prototype. The microcontroller performed:

- LED control
- Sensor data acquisition
- Absorbance calculation
- Calibration processing
- Digital display output

The OLED/LCD display provided direct output of:

- TPC values
- DPPH antioxidant values
- Absorbance readings

**Power System**

The prototype was powered using a rechargeable battery system, allowing portable operation without continuous external power supply. The device could also be operated using USB power during laboratory testing.

**Software Architecture**

**Herbal QC Processing System**

The embedded software was developed using Arduino IDE and implemented the following functions:

- Blank calibration
- Sample absorbance measurement
- TPC calculation
- DPPH calculation
- Display of analytical results

The firmware converts optical sensor readings into absorbance values and applies pre-programmed calibration equations to generate final analytical output.

**Pre-Programmed Assay Modes**

Two assay modes were implemented in the prototype:

1. **TPC Mode** – for Folin–Ciocalteu assay at 760 nm
2. **DPPH Mode** – for antioxidant analysis at 520 nm

The user selects the required assay mode through the control buttons, and the corresponding LED and calibration model are activated automatically.

**Digital Output**

The developed SPHQCC prototype displays:

- Absorbance values
- TPC values (mg GAE/g)

- DPPH antioxidant activity

through the OLED/LCD interface. The digital display reduces manual calculations and improves ease of operation.

**Key Features and Specifications**

| Feature | Specification |
|---|---|
| **Device Type** | Prototype Smart Portable Herbal QC Colorimeter |
| **Optical System** | LED–Photodiode based |
| **LEDs Used** | 520 nm and 760 nm |
| **Detector** | BPW34 Photodiode |
| **Processing Unit** | ESP32 / Arduino |
| **Display** | OLED/LCD |
| **Assays Implemented** | TPC and DPPH |
| **Power Supply** | Rechargeable battery / USB |
| **Sample Holder** | Standard cuvette chamber |
| **Measurement Principle** | Beer–Lambert Law |
| **Application** | Herbal quality analysis |
| **Prototype Nature** | Preliminary analytical prototype |


**Figure :Internal Optical and Electronic Assembly of the Smart Portable Herbal Quality Control Colorimeter (SPHQCC) Prototype.**

Figure : Optical Filter Wheel and Wavelength Selection Assembly Integrated within the SPHQCC Prototype.


**Figure : Digital Display Interface Showing TPC Analysis Mode of the Developed SPHQCC Prototype.**


Figure :Digital Display Interface Showing DPPH Antioxidant Assay Mode of the SPHQCC Prototyp.


**Figure : Internal Printed Circuit Board (PCB) and Signal Processing Circuitry of the Developed SPHQCC Prototype.**

## CONCLUSION

The present research work focused on the development of a Smart Portable Herbal Quality Control Colorimeter (SPHQCC) for preliminary estimation of Total Phenolic Content (TPC) and DPPH antioxidant activity in herbal samples. The developed system was designed as a compact, portable, and cost-effective prototype integrating LED–photodiode optical sensing with Arduino/ESP32-based digital processing.

The work was carried out systematically through stages including literature review, prototype design, optical system development, hardware assembly, software programming, calibration, herbal sample analysis, and comparative validation using a standard UV–Visible spectrophotometer.

The developed prototype was fabricated by modifying a conventional digital photo colorimeter and integrating:

- Multi-wavelength LED system

- BPW34 photodiode sensor
- LM358 signal amplification circuit
- ESP32/Arduino microcontroller
- OLED/LCD digital display

The device was designed for implementation of:

- Folin–Ciocalteu method for TPC estimation at approximately 760 nm
- DPPH antioxidant assay at approximately 520 nm

Calibration curves prepared using gallic acid and ascorbic acid standards demonstrated good linearity and reproducible analytical response. The SPHQCC showed close agreement with the reference UV–Visible spectrophotometer during preliminary validation studies.

Herbal sample analysis using Green Tea (*Camellia sinensis*) and Turmeric (*Curcuma longa*) demonstrated that the developed prototype was capable of producing TPC and antioxidant activity values comparable to those obtained using the standard laboratory instrument, with only minor variation observed between the two systems.

The developed prototype also demonstrated several practical advantages including:

- Portability
- Reduced cost
- Battery operation
- Simplified analysis
- Digital result display
- Reduced dependence on laboratory infrastructure

Overall, the study successfully demonstrated the feasibility of developing a prototype Smart Portable Herbal Quality Control Colorimeter for preliminary herbal quality analysis.

Based on the findings of the present study, the following conclusions were drawn:

1. The Smart Portable Herbal Quality Control Colorimeter (SPHQCC) prototype was successfully designed and developed using LED-based optical sensing and microcontroller-based digital processing.
2. The developed prototype was capable of performing preliminary Total Phenolic Content (TPC) estimation and DPPH antioxidant analysis in herbal samples.
3. The calibration studies using gallic acid and ascorbic acid standards demonstrated acceptable linearity and analytical response within the selected concentration ranges.
4. Comparative analysis showed that the SPHQCC prototype produced results closely comparable to the reference UV–Visible spectrophotometer, indicating good preliminary analytical reliability.
5. The developed system demonstrated significant practical advantages such as portability, low cost, ease of operation, reduced analysis time, and battery-powered operation.
6. The prototype provides a proof-of-concept platform for portable herbal quality analysis and demonstrates the potential application of LED–photodiode optical sensing systems in herbal and nutraceutical quality control.
7. Although the present work is limited to prototype-level development and preliminary validation, the results indicate strong potential for future optimization and further analytical improvement.
8. The developed SPHQCC prototype may be useful for:

- Academic laboratories

- Herbal research studies
- Preliminary field-level herbal analysis
- Educational and demonstration purposes

## FUTURE SCOPE

The present work represents a prototype-level development of a Smart Portable Herbal Quality Control Colorimeter. Further improvements may enhance the analytical accuracy, portability, and practical applicability of the system.

Future work may include:

- Integration of advanced machine learning algorithms
- Development of mobile application connectivity
- Cloud-based data storage and analysis
- Addition of multiple herbal assay modes
- Improvement in optical alignment and sensor sensitivity
- Miniaturization of hardware components
- Large-scale validation using diverse herbal samples
- Commercial prototype development for industrial applications

The developed prototype provides a strong foundation for future research in portable herbal analytical instrumentation.

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