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Phytochemical Profiling and Morphological Characterization of *Hibiscus rosa-sinensis* and *Clitoria ternatea* Flower Petal Extracts

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Phytochemical profiling and morphological characterization of flower petals provide valuable insights into their bioactive constituents and potential therapeutic applications. This study investigates the phytochemical composition and morphological characteristics of the petals of Hibiscus rosa-sinensis (Hibiscus) and Clitoria ternatea (Blue Pea), two widely recognized plants with significant cultural and medicinal importance. The petals of both flowers were subjected to solvent extraction using methanol, and the resulting extracts examined the availability of key phytochemicals, A few examples are alkaloids, flavonoids, tannins, saponins, phenolic compounds, and anthocyanins, was assessed. The morphological features, such as petal color, shape, and size, were also documented and compared. Phytochemical screening revealed the presence of various bioactive compounds, with Hibiscus rosa-sinensis showing a higher concentration of anthocyanins and flavonoids, while Clitoria ternatea demonstrated abundant alkaloids and saponins. The morphological analysis indicated distinct petal shapes and vibrant colors, which are characteristic of their respective species. These findings suggest that both Hibiscus rosa-sinensis and Clitoria ternatea petals are rich in bioactive compounds, which may account for their traditional use in herbal medicine and food applications. The study provides a foundation for further investigation into the therapeutic potential and commercial utilization of these flowers. This article aims to promote the incorporation of edible flowers into consumer diets and the food industry, emphasizing the ability to serve as a plentiful supply of nutraceutical substances.

Keywords: Ciltoria ternatea; Hibiscus rosa-sinensis; Phytochemicals; SEM; XRD.

Hibiscus rosa-sinensis, a species belonging to the Malvaceae family, is commonly referred to as Chinese Hibiscus or tropical Hibiscus.*Top of FormBottom of Form*In India, *Hibiscus rosa-sinensis* flowers are traditionally used as a garnish for dishes, and ingredients in tea, sorbet, and jelly. Medicinally, *Hibiscus rosa-sinensis* is known for its potential to lower blood pressure, reduce cholesterol levels, and aid in weight loss. It is frequently implemented to treat disorders like high blood pressure in traditional Chinese medicine, fever, and liver disease.

Clitoria ternatea, a member of the Fabaceae family, is a medicinal plant known for its

pink, white, and blue flowers. Commonly referred to as *Aparajita* or *butterfly pea*, it has gained significant attention for its medicinal properties, including antibacterial, anti-inflammatory, and antidepressant effects. *Clitoria ternatea* is also recognized for its cognitive-enhancing properties, acting as a brain tonic and immunity booster, with antioxidant properties found in both natural and synthetic sources. The use of natural sources is preferred due to their lower toxicity and minimal side effects. Tocopherol, ascorbic acid, â-carotene, and uric acid are a few such antioxidants. Which are prevalent in fruits and green vegetables, offer non-enzymatic protection against lipid peroxidation in biological systems. These antioxidants mitigate oxidative stress by donating electrons and hydrogen atoms, thereby neutralizing reactive species. Both *Hibiscus rosa-sinensis* and *Clitoria ternatea* contain various phytochemicals that are increasingly recognized for their health-promoting properties, aiding in cellular maintenance and repair across tissues and organs. Phytochemicals, which are compounds derived from plants that exert specific health effects, are not essential nutrients like minerals, vitamins, carbohydrates, proteins, or lipids. However, they have been associated with health benefits, Particularly, phenolic compounds, carotenoids, organic acids, and bioactive constituents such as saponins and sterols are of significant interest. The role of phytochemicals in public health is of growing interest worldwide, and they are viewed by researchers, industries, and policymakers as valuable tools for improving public health. Despite their increasing recognition, the mechanisms and safety of phytochemicals remain poorly understood, warranting further validation and the establishment of scientific databases to address safety concerns and functional mechanisms. Although genetic-based studies propose mechanisms for the health benefits of phytochemicals, there is still a need for more comprehensive research, as discussed in *Bioavailability of Phytochemicals,* [1]

The main aim of this research was to assess the phytochemical substance of *Hibiscus rosa-sinensis* and *Clitoria ternatea* employing various analytical techniques, alongside a comprehensive morphological analysis of the flower petals utilizing X-ray diffraction (XRD) and scanning electron microscopy (SEM).

MATERIALS AND METHODS

Chemicals

Anhydrous aluminum chloride, ferric chloride, sodium nitrate, gallic acid, quercetin, Folin-Ciocalteu reagent, methanol, and distilled water were used in the research. All additional chemical and solvent used was of analytical standards.

Collection of plant specimens

The flowers of *Hibiscus rosa-sinensis* (Malvaceae) and *Clitoria ternatea* (Fabaceae) were harvested from plants cultivated in the garden of the Horticulture Department, BBAU, Lucknow, between July and September. Flower selection was based on petal color and size, with mature red flowers consisting of five petals chosen from *H. rosa-sinensis*. and dark blue flowers from *C. ternatea* were harvested, with the calyx and other floral parts being removed prior to collection.

Preparation of flowers petal extract

The extraction method and solvent selection were based on the specific phytochemicals present in the flowers and the intended analysis. Healthy, mature, and fresh flower petals were first thoroughly washed with fresh water for 5 minutes,[2] The petals were then finely crushed into small pieces. One gram of the fresh material was immersed in 10 mL of 60% ethanol solution. The combination was placed in a rotary shaker set at 100 rpm and maintained for 3 hours at room temperature. Subsequently, the extract was shaken. at 5000 rpm for 15 minutes, and the resulting the leftover fluid was aliquoted and kept at 4°C in a refrigerator.,[3] Whatman No. 1 filter paper was utilized to filter the extract. to eliminate any particulate matter, the residue was stored in an airtight container for subsequent analysis.[4]

Qualitative Phytochemical screening

Phytochemical screening of plant edible flower petals extract involves identifying and quantifying the bioactive compounds present in the extract. Ten groups of compounds are present, as mentioned below. was determined using various analytical techniques.[5-6]

Test for tannin 1ml of *Clitoria ternatea* (Blue Pea) extract and 1 ml of *Hibiscus rosa-sinensis* extract were each combined with 1 ml of 5% ferric chloride (FeCl*f*) solution. The development of dark blue and greenish-black

coloration upon mixing This suggests the presence of tannins in the extracts.

Saponin 1 ml of *Clitoria ternatea* (Blue Pea) extract and 1 ml of *Hibiscus rosa-sinensis* extract were each mixed with After adding 1 ml of distilled water, the extract was stirred for 15 minutes. The formation of a 1 cm layer of foam upon shaking serves as an indicator of the presence of saponins in the extract.

Quinone 1 ml of *Clitoria ternatea* (Blue Pea) extract and 1 ml of *Hibiscus rosa-sinensis* extract were each treated with 1 ml of concentrated sulfuric acid (H, $SO_{,,}$). The development of a red hue upon reaction indicates the presence of quinones in the extracts.

Flavonoid 1 ml of *Clitoria ternatea* (Blue Pea) extract and 1 ml of *Hibiscus rosa-sinensis* extract were each treated with 1 ml of 2N sodium hydroxide solution. (NaOH). The development of a yellow color upon reaction shows the presence of flavonoids in the extracts.

Glycosides 1 ml of *Clitoria ternatea* (Blue Pea) extract and 1 ml of *Hibiscus rosa-sinensis* extract were each using 3 ml of chloroform, Afterward, 10% was added. ammonium solution. The development of a pink color upon reaction indicates the presence of glycosides in the extracts.

Alkaloids 1 ml of *Clitoria ternatea* (Blue Pea) extract and 1 ml of *Hibiscus rosa-sinensis* extract were dissolved in a small volume of hydrochloric acid (HCl). Alkaloids are present in the extracts when a crystalline precipitate develops as a result of the reaction.

Phenols 1 ml of *Clitoria ternatea* (Blue Pea) extract and 1 ml of *Hibiscus rosa-sinensis* extract were each mixed with After adding 2 ml of distilled water, a few drops of 10% ferric chloride (FeCl*f*) solution. The formation of blue-green coloration upon reaction indicates the presence of phenolic compounds in the extracts.

Coumarins 1 ml of *Clitoria ternatea* (Blue Pea) extract and 1 ml of *Hibiscus rosa-sinensis* extract were each Applied to 1 ml of sodium hydroxide (10%) (NaOH) solution. The development of a yellow color upon reaction demonstrates that there is of coumarins in the extracts.

Anthocyanin and betacyanin 1ml of *Clitoria ternatea* (Blue Pea) extract and 1 ml of *Hibiscus rosa-sinensis* extract were each treated with 1ml of sodium hydroxide (NaOH) solution and subjected to heat for five minutes at 100°C. The development of a bluish-green color signifies the presence of anthocyanins, while the formation of a yellow color suggests the existence of betacyanins in the extracts.

Quantitative Assessment

Total polyphenol content (TPC) quantification

TPC, or total polyphenol content, determines using a widely employed spectrophotometric method Determined by the lowering of the Folin-Ciocalteu reagent,[7-8] To perform the assay, 5 ml of distilled water were incorporated with 1 ml of the sample extract, followed by 0.5 ml of Folin-Ciocalteu reagent was added. The mixture is shaken and allowed to rest for 5 minutes. Subsequently, 20% sodium carbonate solution (1.5 mL) is added, and the reaction is incubated for 2 hours in the dark. After incubation, the absorbance is measured at 750 nm. The procedure was carried out at least three times, and the mean ± standard deviation is the result. computed with the aid of the subsequent formula.

$$TPC = C \times (v/m)$$

Where, C represents the concentration of phenolic compounds, m is the sample of mass, and v is the extract's volume.

Total flavonoid content (TFC) quantification

Using aluminum chloride, (AlCl*f*) the total flavonoid content (TFC) has been determined. the total flavonoid content (TFC) has been determined. colorimetric method,[9] a widely used spectrophotometric techniques based on the formation of a flavonoid-$AlCl_3$ complex,[10] The assay is performed out by mixing 1 mL of sample with 4 mL of distilled water, then adding 0.2 mL of 5% sodium nitrate. ($NaNo_2$) solution. The mixture is shaken, and then0.3 mL of 10% aluminium chloride ($AlCl_3$) solution is added. The volume is adjusted to 10 mL with distilled water, resulting in the formation of an orange-yellow color. The absorbance is measure at 510 nm ,[11] The process was presented as mean ± standard deviation, which was calculated using the equation below.

$$TFC = C \times (v/m)$$

where C represents the concentration of flavonoids, m is the sample of mass, and v is the extract's volume.

Structural analysis by SEM and XRD

(SEM) for scanning electron microscopy

Images from scanning electron microscopy (SEM) have been recorded with a JEOL JSM 6490 device.Top of FormBottom of Formmanufactured in Japan. Prior to imaging, the flower petal specimens underwent a dehydration process utilizing a graded sequential concentration of acetone: 30%, 50%, 70%, 90%, and 100% (dry acetone). Following dehydration, the specimens were subjected to critical point drying, [12] to preserve their structural integrity. subsequently drying, the samples were gently set on aluminum stubs. Using adhesive tape with two sides,[13-14]To enhance electrical conductivity and prevent charging artifacts, The samples were then coated a very thin layer of gold using a sputter coater. SEM micrographs were obtained under conditions of high vacuum with a 5 kV accelerating voltage, enabling high-resolution imaging of the flower petals' surface morphology.

X-ray diffraction (XRD)

X-ray diffraction (XRD) analysis yields essential Specifications of structure that have a direct influence on the material's observed characteristics,[15] It offers insights into various structural characteristics, including phase identification, Texture, average crystallite size, crystallinity, strain, and crystallographic defects. XRD is particularly useful for identifying the crystalline phases of a sample and determining unit cell dimensions. In this study, petal extract powders were precisely placed into Glass crucibles that are rectangular and analyzed using X-ray diffraction. The samples were irradiated with an X-ray beam (8 keV) created by a Mini Flex-II Desktop X-ray diffractometer (Japan), which is adapted with a è-è goniometer. and operated at 25 mA and 30 kV, using Cu Ká filtered radiation. The 2è scanning range was set from 6° to 45° to capture all significant diffraction peaks from the sample crystallites, with a scan speed of 3°/min. Crystallite size was determined using the Warren-Averbach method, which applies Fourier theory to analyze the broadening of X-ray reflections. The crystallite size distribution was computed using an established analytical function for crystal size distribution

RESULTS AND DISCUSSIONS

The quanlitative phytochemical screening of *Hibiscus rosa-sinensis* and *Clitoria ternatea* flower petal extracts were conducted to identify and quantify various phytochemical constituents, including tannins, saponins, quinones, flavonoids, glycosides, alkaloids, phytosterols, phenols, coumarins, anthocyanins, and betacyanins, using established methods,[16] The phytochemical analysis results indicated the presence of (+) and absence (-) of specific compounds in the petal extracts of both species. In *Hibiscus rosa-sinensis*, the extract contained tannins, saponins, flavonoids,

Table 1. Shows the presence and absence of Phytochemical compounds of Hibiscus rosa-sinensis and Clitoria ternatea flower petals extract

Phytochemical Compounds	Observation	Hibiscus rosa-sinensis	Clitoria ternatea
Tannin	(+) greenish black color	+	_
Saponin	(+) foam formation	+++	+
Quinone	(-) green color	_	_
Flavonoid	(+) Yellow color	++	_
Glycosides	(+) Pink color	++	++
Alkaloids	(+) Orange or Red	_	+
Phytosterols	(+) Bluish Green	_	+
Phenols	(+) green color	+	+
Coumarins	(-) Yellow color	_	_
Anthocyanin	(+) yellow color	+++	+++
Betacyanin	(-) Red color	_	_

+++ = substantial quantity (positive in five minutes); ++ = modestly (positive after five minutes); + = trace number (positive after ten minutes.). And - = (Absence)

glycosides, and anthocyanins, as indicated in Table 1. In contrast, *Clitoria ternatea* extract was found to contain saponins, glycosides, alkaloids, phytosterols, and anthocyanins. The presence of anthocyanins in both extracts is particularly noteworthy, as these compounds are associated with antioxidant activity, suggesting potential health-promoting properties,[17] These results correlate with previous research. on the phytochemical profiles of these flowers and provide a basis for the further exploration of their potential applications in natural product development with therapeutic benefits. The findings are in agreement with previous research on the same species, which also reported the presence of these phytochemicals. [18-19]

Table 2 compared Hibiscus rosa-sinensis and Clitoria ternatea, emphasizing significant differences in Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). Hibiscus rosa-sinensis shows low standard errors (±0.129 for TPC and ±0.012 for TFC), indicating precise and consistent measurements. Clitoria ternatea has higher standard errors (±0.4445 for TPC and ±0.057735 for TFC), reflecting more variability in phenolic and flavonoid concentration between samples.

Table 2. Presents TPC and TFC, total phenolic and flavonoid contents in *Hibiscus rosa-sinensis* and *Clitoria ternatea*

Sample	TPC (mg GAE/g)	TFC(QE mg/g)
Hibiscus rosa-sinensis	035.82±0.129	061.59±0.012
Clitoria ternatea	0.65116±0.44455	0.31333±0.057735

Data represented as mean ± SE (n=3)

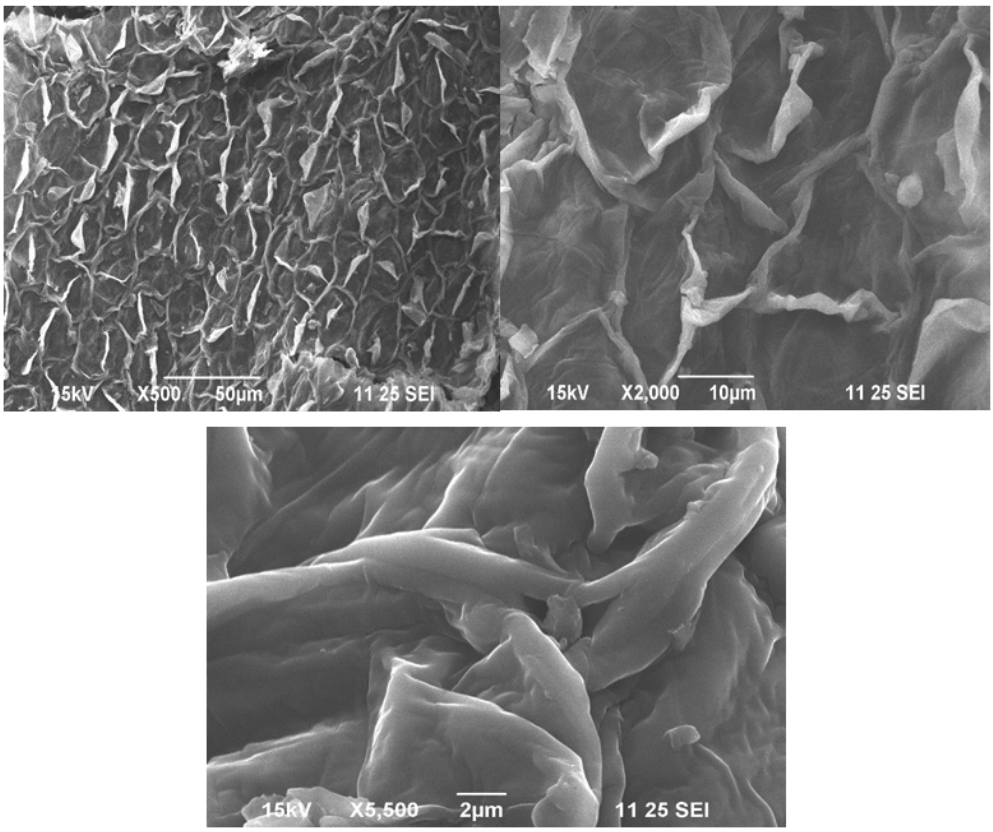

Fig. 1. Shows SEM Micrographs of Hibiscus rosa-sinensis

This study suggests that Hibiscus rosa-sinensis has a higher concentration of flavonoids, these compounds are recognized for their antioxidant and anti-inflammatory activities. This study found that the extract of *Clitoria ternatea* exhibits a higher total phenolic content (TPC) compared to total flavonoid content (TFC). This observation is consistent with the general

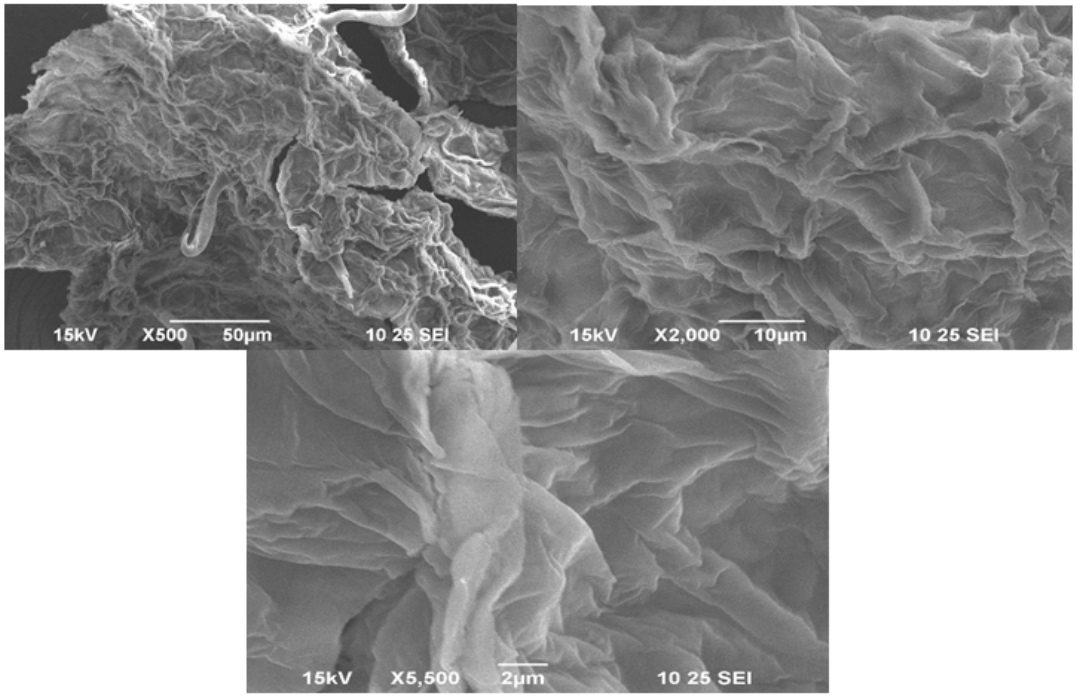

Fig. 2. SEM micrographs of Clitoria ternatea

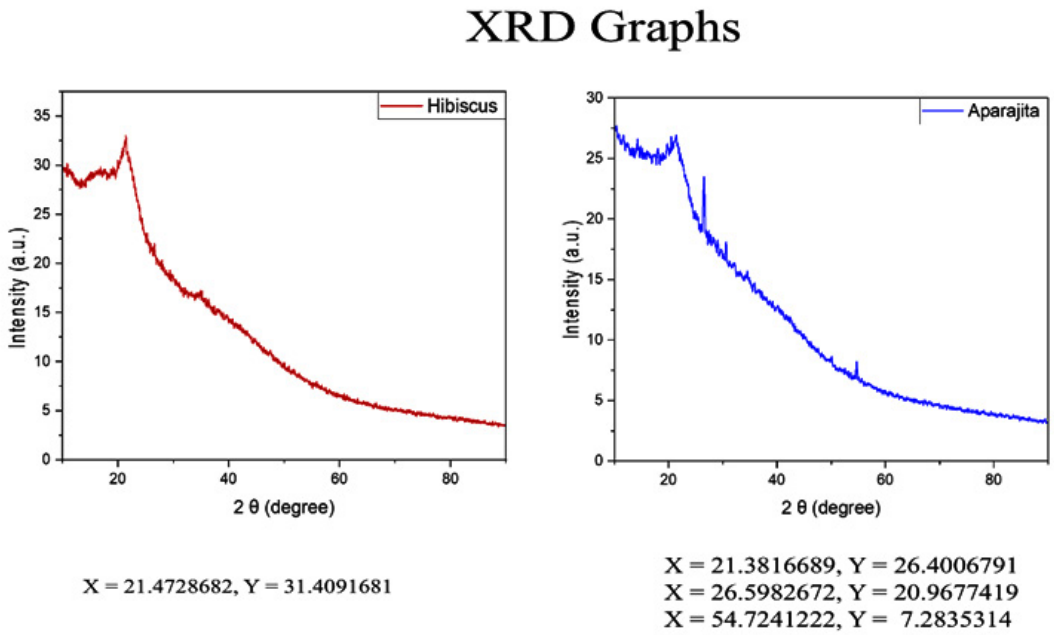

Fig. 3. X-ray diffractograms of Hibiscus rosa-sinensis and Clitoria ternate

trend, where the total phenolic content (TPC) is typically greater than the total flavonoid content (TFC). However, a comparison with previous studies reveals discrepancies in the observed values for both TPC and TFC. Specifically, the TPC value in this study is 0.65116 ± 0.44455 mg GAE/g, which is considerably lower than the TPC value of 64.8 ± 0.1 mg GAE/g reported in a similar study that extracted *Clitoria ternatea* leaves using 70% methanol. Several factors could contribute to this difference, including variations in extraction methods, plant material, and environmental conditions, among others, [20] The total quantity of phenolic of *Hibiscus* was determined to be 35.82 ± 0.129 mg/g GAE (Table 2). The plant's flavor, color, and protective qualities are all determined by the presence of polyphenolic chemicals, [21] The total flavonoid content of Hibiscus was found to be 61.59 ± 0.012 mg QE/100 g dry weight (Table 2). The estimation of total flavonoid content provides valuable insight into the plant's antioxidant, [22] these findings indicate that Hibiscus is a more potent source of phenolic and flavonoid compounds compared to Blue Pea (Aparajita), and thus may hold greater potential for applications in antioxidant-related therapies and functional foods. These phenolic and flavonoid compounds improve human health by mitigating oxidative damage, providing protection from a variety of diseases associated with oxidative stress, including cardiovascular disease, cancer, and neurological ailments.[23]

The first illustration illustrates a series of micrographs of Hibiscus rosa-sinensis taken at various magnifications to demonstrate reliable morphological aspects of its surface structure. At 500× magnification, the surface has a honeycomb-like porous structure made of connected irregular gaps or cells. The appearance of sheet-like walls and blank spaces indicates that the material is foamed or porous, which is a commonly morphology in polymeric foams. At a magnification of 2000×, the structure resembles crumpled, sheet-like, with wrinkled walls or layers. These layers appear to be interconnected illustrating the surface microstructure's complexity and texture. At 5500× magnification, the micrograph indicates a wrinkled, fibrous, and sheet-like structure. The surface reveals significant folds, wrinkles, and overlapping layers, indicating a highly organized and possibly hierarchical fiber nature. The above results collectively shows that Hibiscus rosa-sinensis has a multi-scale porous architecture with high structural complexity, which might prove useful for applications involving surface area, porosity, or sorption.

Figure 2 illustrates micrographs of Clitoria ternatea at progressively greater magnifications, demonstrating its unusual surface shape. At 500× magnification, the surface has an uneven, stratified, and wrinkled structure, indicative of materials with large surface areas. The observed morphology indicates a potentially positive texture for applications that require improved surface interaction. At 2000× magnification, the structure reveals thin lamellar layers that resemble piled or crumpled sheets. This layered construct indicates a complex microstructure with enhanced surface characteristics and potential flexibility. At 5500× magnification, the image indicates a flexible, sheet-like microstructure with a rough and slightly granular appearance in certain areas. Compared to the 500× image, the greater magnification clearly displays finer structural elements, including subtle granularity and increased surface complexity. These microstructural characteristics show that Clitoria ternatea has a multi-layered, high-surface-area morphology, which could be beneficial for adsorption, catalysis, and bio-interface applications.

The x-ray diffraction (XRD) patterns of Hibiscus rosa-sinensis and *Clitoria ternatea* provide insight into the state of phase and crystal framework composition of these materials. The degree of diffraction angle (2è) is expressed in degrees by the X-axis., while the Y-axis corresponds to the intensity of the X-rays that were diffracted, expressed in arbitrary units (a.u.). These diffraction peaks are indicative of specific interplanar spacings, as defined by Bragg's Law. The 2è values in the graphs span approximately from 10° to 90°.For *Hibiscus rosa-sinensis*, a prominent peak is observed at a 2è value of approximately 21.47°, with an intensity of 31.41 a.u. This peak corresponds to a specific diffraction angle, associated with the crystallographic planes of the material. For *Clitoria ternatea*, two significant peaks are noted: one at 21.38° with an intensity of 26.40 a.u., and another at 26.60° with an intensity of 20.97 a.u. The broad nature of the diffraction

peaks in both XRD patterns suggests that the materials exhibit low crystallinity, consistent with the presence of an amorphous phase. The relatively less sharp peaks further support this observation, indicating that both *Hibiscus rosa-sinensis* and *Clitoria ternatea* are predominantly amorphous or partially crystalline in nature.

CONCLUSION

The phytochemical analysis of flower petals identified a broad spectrum of bioactive compounds, including flavonoids, alkaloids, saponins, and glycosides. Hibiscus rosa-sinensis is notably abundant in saponins, anthocyanins, and glycosides, with moderate concentrations of flavonoids, phenolic compounds, and tannins. Conversely, Clitoria ternatea contains moderate levels of saponins, glycosides, and alkaloids, with trace amounts of flavonoids and phytosterols. This phytochemical profile suggests that Hibiscus rosa-sinensis possesses a higher antioxidant potential, given the significant levels of both phenolic compounds and flavonoids, which are known for their strong antioxidant properties and associated health benefits, such as anti-inflammatory and anti-cancer effects. In contrast, Clitoria ternatea, while rich in certain bioactive compounds, has comparatively lower concentrations of these antioxidant-rich compounds. Therefore, based on the data presented, Hibiscus rosa-sinensis could be considered a more potent source of antioxidants than Clitoria ternatea, making it potentially more beneficial for health-promoting purposes. Additionally, the morphological characterization of flower petals provides essential insights into their structural attributes, functions, and diversity. This study adds to the growing body of research on the phytochemical composition and morphological characteristics of flower petals. Underscoring their potential as a natural resource for promoting human health and well-being. Further exploration of these compounds and structural characteristics could pave the way for innovative applications and the sustainable utilization of flower petals in various industries.

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Conflict of Interest

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This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

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Not Applicable.

Authors' Contribution

Sunita Mishra: Visualization, supervision; Zia Parveen: Conceptualization, Methodology, Writing – Original Draft; Narendra Kumar: Review & Editing; Kuril Sanjeet Babulal: Data collection; Rajani Singh: Guidance in laboratory analysis.

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