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PHYTOCHEMICAL SCREENING OF *CURCUMA CAESIA* AND *CLITORIA TERNATEA*

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Abstract

Curcuma caesia and *Clitoria ternatea* commonly known as black turmeric and butterfly pea flower belongs to Zingiberaceae and Fabaceae family. Both are medicinal plants with essential therapeutic activity because of their diverse phytochemical profiles. This review solidifies current research on their bioactive compounds such as flavonoids, alkaloids, terpenoids, phenolics, emphasizing their pharmacological relevance. The bioactive compounds of *Curcuma caesia* and *Clitoria ternatea* are extracted using Soxhlet extraction technique which also ensures the high yield and purity. And this review also highlights the microbial activities against pathogens like *Staphylococcus aureus and Escherichia coli*, following mechanisms such as disruption of microbial cell membranes and inhibition of essential enzymatic pathways. Apart from antimicrobial activity both plants also exhibit strong antioxidant activity demonstrated by DPPH radical scavenging assays, as it shows their capability to neutralize free radicals and reduce oxidative stress. It also shows properties like anti-inflammatory, wound healing, antiviral, antiulcer. The unification of these bioactivities acts as promising candidates for pharmaceutical, cosmetic and nutraceutical formulations. This review merge current insight on their chemical composition, biological properties and therapeutic potential.

Keywords: *Curcuma caesia*, *Clitoria ternatea*, antimicrobial activity, Antioxidant activity, DPPH assay, Soxhlet extraction

1. INTRODUCTION

Medicinal plants have been used from ages in traditional system of medicines, and they are known as the primary source of the food chain as they are rich in nutrients and biochemical compounds and provide beneficial effects [1]. Nowadays, medicinal plants are gaining a huge attention in modern medicine because their worldwide acceptance, safety and affordability [2]. *Clitoria ternatea* also known as Butterfly Pea, is a type of an herbaceous perennial climber plant and belongs to the Fabaceae family. It is a tropical flower that can be easily found in gardens and also in the wild and is the native plant of Zimbae, Ghana, Guinea, Malaysia, Indonesia. It shows various health benefits like antidiabetic, antidepressant, memory enhancing, antioxidant because of the presence distinct bioactive compounds like anthocyanins- it also provides blue colour to the flower [3].

Curcuma caesia is also one of the medicinal plants, commonly known as black turmeric or kali haldi and belongs to the Zingiberaceae family. It is very famous among ethnic groups of Nepal and India because of the various medicinal properties. The rhizomes and leaves of Curcuma caesia show the property like antibacterial, antioxidant, anticancer, anti-inflammatory [4].

This review aims to provide a comprehensive overview of the phytoconstituent, biological activities and therapeutic potential of *Curcuma caesia* and *Clitoria ternatea* with particular focus on the antimicrobial and antioxidant property. This review highlights their phytochemistry, extraction, and skin-related applications.

For any tropical formulation skin acts as a physical barrier and protects the body from the external environment by limiting the attack of environmental threats [5]. Plant based ingredients and extracts have been used from ages for skin care purpose. Source include herbs, fruits, flowers, leaves, stem, root.

Plant based bioactive compounds have been an interesting topic for research therefore, *Curcuma caesia* and *Clitoria ternatea* can be the promising candidates for the drug discovery and development, but the use of extract require concentration on the extraction methods and type of solvent used [6].

For the extraction of bioactive metabolites rhizome part of Curcuma caesia is used. And the ethanolic extract shows activity against pathogenic bacteria like E. coli, S. aureus [7].

Curcuma caesia (Black turmeric) and *Clitoria ternatea* (Butterfly pea) are two ethnomedicinal plants that are rich in phytochemicals that have potential dermatological benefits. *Curcuma caesia* contains alkaloids, terpenes, amino acids, carbohydrates, tannins, flavonoids, steroids, proteins and phytochemicals such as camphor, ar-turmerone, borneol, ocimene. And it is used for the treatment of pneumonia, cough, and for cold in children, fever, wounds, antioxidant, antimicrobial, snake and scorpion bite [8]. On the other hand, *Clitoria ternatea* contains anthocyanins, triterpenoids, flavanol glycosides, steroids. And is used in the treatment of body aches, infections, urinogenital disorders, anthelmintic and as an antidote to some animal stings [9].

1.1 PHARMACOLOGICAL ACTIVITY

The various phytochemicals of *Curcuma caesia* such as tannins, terpenoids, flavonoids, alkaloids, phenol, and saponins shows the biological activities like anti-inflammatory, antimicrobial, antioxidant, anticancer [10]. And *Clitoria ternatea* contain various phytochemicals like saponins, carbohydrates, triterpenoids, phenols, steroids, anthocyanins and shows pharmacological effects like antioxidant, antidiabetic, antimicrobial, anti- inflammatory, antipyretic [11].

- **Antimicrobial activity**

Ethanolic extracts of *Clitoria ternatea* were tested for antibacterial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Enterococcus faecalis,* using the well diffusion method. The ethanolic extracts shows significant antibacterial activity, with zones of inhibition. It's ethanolic extracts are especially potent against bacteria that cause urinary tract and wound infections, but studies also indicates that their toxicity increases as the dose goes up, emphasizing the need to approach their medical use with caution. Among the different parts of *Clitoria ternatea,* leaf and root extracts stand out as the most active [12].

The essential oil extracted from *Curcuma caesia* commonly known as black turmeric, was tested for its ability to fight against bacteria using a method where it is measured how well it stops bacterial growth on a petri dish. The test included four types of bacteria that are Gram-negative (*Salmonella typhimurium and Escherichia coli*) that are Gram-positive (*Bacillus cereus and Staphylococcus aureus*). This approach helps us to understand how effective the oil is at inhibiting the growth of these bacteria under controlled lab conditions [13].

- **Antioxidant activity**

The DPPH assay method was used to test the antioxidant activity of *Curcuma caesia* rhizome extracts. It is measured by how well a extract can neutralize harmful free radicals, which are unstable molecules and can damage cells. The DPPH chemical is purple in colour, but when antioxidants are present, they "donate" an electron to DPPH, and results into the change of colour from purple to yellow. The more yellow it becomes, the stronger is the effect.

Amid the different extracts, the ethanol extract indicates the strongest ability to neutralize free radicals. Other extracts like methanol, ethyl acetate, and water also had good antioxidant effects but to a lesser extent [14].

For testing antioxidant activity of *Clitoria ternatea* DPPH assay method is used. After testing leaf and stem extract shows strong antioxidant activity and seed extract shows very low antioxidant activity [15].

- **Anthelmintic activity**

For the study of anthelmintic activity earthworms were used as they are anatomically and physiologically similar with intestinal roundworm parasites. Then the earthworms of similar size are selected and divided into 11 groups and each group containing 6 earthworms. Then the earthworms were released in the different samples such as control, standard drug, ethanol, chloroform extracts. Then the time taken for paralysis and death were observed [16].

- **Anti- asthmatic activity**

Pritesh Paliwal and colleagues explore the bronchodilating effect of *Curcuma caesia*. For testing the anti-asthmatic activity guinea pig were used and exposed to histamine aerosol to trigger bronchospasm and breathing difficulties. And when it is treated with methanolic extract of *Curcuma caesia* the guinea pig shows remarkable protection. And in some aspects shows much better result when compared to standard drug chlorpheniramine maleate [17].

- **Vision protection**

Extracts of *Clitoria ternatea* and eye gels are used in sometimes to treat vision related problems like glaucoma, damage retinas, blur visions, poor night vision, strained eyes. And the antioxidant property of *Clitoria ternatea* also provide protection against the eyes from the free radical damage that is caused by the sun, irritants [18].

- **Anti-inflammatory activity**

The anti-inflammatory activity of Black turmeric was tested using three different methods: a proteinase inhibitory test, a cyclooxygenase activity test, nitrate/nitrite assay. Diclofenac test was used in the proteinase inhibitory test as a standard for comparison. It is done to test how well the Black turmeric prevent the denaturation of proteins. Absorbance was recorded and percentage of inhibition is calculated [19].

- **Antidiabetic activity**

Diabetes mellitus is known as a metabolic disorder and normally it is known as hyperglycaemia. Naturally diabetes is managed by using plant-based compounds that slow down the breakdown of complex carbohydrates into glucose. And inhibition occurs due to key enzymes such as alpha amylase. The presence of phenolic compounds plays an important role in this process. Methanol extract exhibits strong activity against alpha- amylase, while on the other hand n-hexane and water-soluble residue shows about 50% inhibition of the pepsin enzyme [20].

- **Anticancer effect**

The cytotoxic activity of *Clitoria ternatea* flower extracts was evaluated in vitro using the trypan blue dye exclusion method. Petroleum ether extract and ethanolic extract shows dose dependent cytotoxic effect on cells. The petroleum ether extract reduces the cell count by 8% at lowest concentration and shows complete reduction at highest concentration. While on the other hand ethanolic extract shows 80% reduction [21,22].

Both *Clitoria ternatea* and *Curcuma caesia* are two important ethnomedicinal plants with various pharmacological activities. Together, these plants can be considered as valuable candidates for drug discovery and formulation development, especially in skin care.

Table1. Plant extract reported and pharmacological property

Plant	Property/ Use	Extract/ Compound	Observation/ Mechanism	Reference
Curcuma caesia	Antimicrobial and antifungal	Essential oil, methanol and ethanol rhizome extracts	*Inhibits B. subtilis, B. cereus, E. coli, S.aureus, antifungal against S. cerevisiae*	23,24
	Antioxidant	Methanol, ethanol, aqueous extract	Scavenges DPPH, ABTS, nitric oxide, hydroxyl, superoxide radicals; boosts GSH and GR; reduces SGOT, SGPT	25, 26
	Analgesic	Methanol rhizome extract	Reduces pain in acetic acid induced writhing model and hot plate test in mice.	27
	Anti-inflammatory	Methanol and ethanol extract	Reduces granuloma weight, paw edema, protein denaturation, hemolysis and proteinase activity	27,28
	Anticancer/ Antitumor	Methanol, hexane, chloroform extract like Curcuzederone, Ar-turmerone, Ocimin	Cytotoxicity in HepG2 and EAC cells; reduces tumor weight and volume; tubulin and EGFR docking studies confirm anticancer potential	29,30,31
	Antimutagenic	Methanol, ethanol, aqueous rhizome extract	Inhibits mutagen cyclophosphamide using S. typhimurium strains TA98 and TA100	32
	Hepatoprotective and Enzyme modulation	Methanol extract	Enhances ALP, ALT, AST, AChE activity; protects against DEN-induced liver damage	33
	Anthelmintic	Ethyl acetate rhizome extract	Causes paralysis and death of earthworms at high doses	34
	CNS Activity	Ethyl acetate fraction of methanol extract	Exhibits anxiolytic, antidepressant, and memory enhancing effects in rats	35

	Antiasthmatic	Crude extract	Provides bronchodilation, suppresses histamine-induced bronchospasm	36
	COVID- 19 Related	Ocimin (in silico study)	Shows inhibitory effect on SARS-CoV-2 main protease; competitive with nelfinavir	37
Clitoria ternatea	Antioxidant	Aqueous and ethanol flower petal extract	Aqueous extract shows stronger DPPH radical scavenging than ethanol extract; retained antioxidant activity when incorporated in gel	38
	Antibacterial and cytotoxic	Ethanolic extract	Significant antibacterial activity against *S. aureus, E. coli, K. pneumoniae, P. aeruginosa* showed cytotoxicity in brine shrimp lethality assay.	39
	Antioxidant	Flower extract	Flower tea has strong antioxidant properties in ABTS, FRAP, H_2O_2, DPPH assays	40
	Antibacterial/ anticancer	Methanol leaf extract	Shows antibacterial activity, anticancer activity against HL-60 cells; phytochemical screening revealed alkaloids, flavonoids, terpenoids etc.	41
	Anti- biofilm	Anthocyanin- rich fraction from flower extract	Reduced Pseudomonas aeruginosa biofilm formation; significant inhibition of bacterial attachement	42
	Comparative antioxidant/ antidiabetic/ anti-inflammatory	Methanol and ethyl acetate extracts of flowers and leaves	Measured phenolic contents; displayed antioxidant, anti-inflammatory, and antidiabetic activities in vitro	43

2. MATERIALS AND METHODS

2.1 Collection and preparation of plant material

- **Black Turmeric (*Curcuma caesia*)**

Black turmeric is a rhizomatous herb and grows up to 1-1.5 meters in height. The rhizome is oval in shape and is differentiated by its camphoraceous sweet odour and bluish black inner surface and with a dark brown outer layer. The plant bears broad, oval leaves measuring about 25–50 cm in length and 15 cm in width, often arranged in groups of 10–20 per plant and it usually bloom during the monsoon season, particularly in June and July [44].

BLACK TURMERIC

Table2. Reported phytoconstituents with their biological activities

s.no.	Compound	Reference	S. No.	Compound	reference
1.	Aerugidiol	45,46	11.	1,8-Cineole	54
2.	α-Acorenol	47	12.	Curzerenone	55
3.	Alismoxide	48	13.	Curzerene	55
4.	d- Amorphene	49	14.	Camphor	56
5.	Borneol	50	15.	(Z) -7-methoxy-1, 5 dihydrobenzo [c] oxepine	57
6.	Bornyl acetate	50	16.	Xanthorrizol	58
7.	Bicyclo [3.1.0] hexane-3-one	51	17.	Zedoarondiol	58
8.	1,1,4,4-Tetramethyl-2,3-tetralindione	51	18.	Zerumin B	58
9.	Linalool	52	19.	Curcumin	58
10.	Bornylene	53	20.	Amadannulen	58

- ***Clitoria ternatea*** **(Butterfly Pea)**

Clitoria ternatea also known as butterfly pea, belongs to the Fabaceae family and it is commonly grown in Southeast Asia and other tropical locations [59]. *Clitoria ternatea* contains various kinds of metabolites, such as pentacyclic triterpenoids like taraxerol, taraxerone, ternatins, alkaloids, flavonoids, saponins, tannins, and anthocyanins [60]. The extracts of Clitoria ternatea is mainly used as an ingredient in Medhya Rasayana- a rejuvenating herbal formulation used for the treatment of various neurological disorders [61,62].

CLITORIA TERNATEA

Table3. Reported phytoconstituents and their function

Plant part	Major constituents	Reported function	Reference
leaf	Crude fibre (21.5%), protein (21.5-29%), clitorin, kaempferol derivatives, aparajitin, beta- sitosterol	Nutritional value (fibre, protein), antioxidant, anti- inflammatory, cardioprotective (flavonoids, sterols)	63
Root	Taxaxerol, taxaxerone, flavanol glycosides, starch, tannins, resin, amino acids (glycine, alanine, valine, leucine, aspartic acid, glutamic acid, arginine, histidine)	Anti- inflammatory, antimicrobial, nutritive, metabolic regulation	64,65
Seed	Trypsin inhibitors, mucilage, delphinidin-3,3,5-triglucoside, p- hydroxycinnamic acid, flavonol-3-glycoside, adenosine, hexacossanol, anthoxanthin glycoside, oligosaccharides, sterols, alkaloids, saponins, tannins, proteins, flavonoids, phenolics	Antinutritional (trypsin inhibitors), colouring agent (delphinidin dye), antioxidant, antimicrobial, anti-inflammatory, proteins, sterols, flavonoids	66,67

Flower	Cyclotides, phenolic acids, flavones, flavanols, anthocyanins, flavanol glycosides (kaempferol3-rutinoside, quercetin 3- rutinoside, kaempferol 3-glycoside, quercetin 3- glycoside)	Antioxidant, antimicrobial, anticancer, anti- inflammatory, wound healing, anti-aging (collagen protection)	63

2.2 Extraction

Soxhlet extraction method is known as a continuous solvent extraction method and it uses solvents at ambient pressure and temperature [68]. For the extraction of plant material is crushed with the help of mortar and pestle [69]. In the Soxhlet extraction process, the sample is first placed inside a thimble made of filter paper, which is then positioned in a glass extraction chamber. This chamber is equipped with a siphon tube and an inlet tube, and a water-cooled condenser is attached at the top. The assembly is connected to a round-bottom flask containing the solvent, which is heated using a water or sand bath. As the solvent vaporizes, it travels through the inlet tube to the condenser, where it condenses and drips onto the solid sample, dissolving the desired compounds. When the liquid reaches the top of the siphon tube, it flows back into the flask, carrying the extracted substances. This cycle repeats continuously, allowing efficient extraction. After the procedure, heating is stopped, and the solvent is recovered by distillation, leaving the concentrated extract behind [70].

3. PRELIMINARY PHYTOCHEMICAL SCREENING

The concentrated extracts were used in qualitative test for the identification of various phytochemicals constituents as per standard procedures [71]. Qualitative phytochemical tests are used because of their simplicity, cost-effectiveness, and accessibility. These tests provide necessary information on phytochemical profiles and can provide more detailed studies [72]. Phytochemistry is a field of study with the primary goal of identifying the chemical components of plants. Phytochemistry is a field of study whose primary goal is to identify the chemical components of plants [73].

The major tests and their principles are as follows:

3.1. Molisch Test

The extract was taken and 1ml of Molisch reagent (α-naphthol in ethanol) is added then a few drops of strong sulfuric acid is added, and then the mixture is gently agitated [74].

3.2 Drangondorff's Test

The extract is mixed in 5ml of distilled water and 2M hydrochloric acid is added until the occurrence of acidic reaction then 1ml of Drangondorff's reagent is added [75].

3.3. Benedict's Test

For Benedict's test two solutions are required Benedict's reagent requires two solutions. Solution A is made by addition of 173gm of sodium citrate and 100gm of sodium carbonate in 800ml of distilled water, then the solution is boiled until the solution becomes clear. Solution B is prepared by adding 17.3gm of copper sulphate in 100ml of distilled water. Then the extract is added followed by 5 mL of Benedict's reagent and the mixture is then boiled for upto 5 min [76].

3.4. Liebermann-Burchard Test

a) To plant extract 10 ml chloroform is added and then 2ml of this filtrate is taken and in that 2ml acetic anhydride and conc. H2SO4 is added. Blue green ring indicates the presence of steroids.

b) 2 ml of acetic anhydride was added to the extract and then 2 ml of H2SO4 is added. The colour changes from violet to blue or green indicated the presence of steroids [77].

3.4. Test for Flavonoids and Glycosides

To the plant extract, 10 mL of ethanol was mixed and filtered. Then 2mL of the filtrate, concentrated HCl, and magnesium ribbon were mixed together. The formation of a pink or red color indicates the presence of flavonoids. And the Addition of 1 mL of distilled water and NaOH to the extract, the formation of a yellowish colour indicates the presence of glycosides [78].

3.5. Test for Anthraquinones

To the extract few drops of 10% ammonia solution were added to 1mL. The formation of a pink precipitate was taken as a positive sign for the presence of anthraquinones

And the Sodium hydroxide is added to the extract results and if the formation of a yellow coloration occurs, which then turns colourless after addition of dilute acid, indicates the presence of flavonoids [79].

3.6. Froth / Foam Test (Saponins)

The extract is taken and Shaked vigorously with 5ml distilled water for 15 minutes. Formation of froth or foam formation indicates the presence of saponins. Stable foam that lasts for 10–15 minutes confirms the presence of saponins [80].

3.7. Gelatin Test

The extract is taken in test tube and 1-2ml of 1% gealtin solution containing NaCl is added and if results in the formation of white precipitate confirms the presence of tannins [81].

3.8 Salkowski Test (Steroids / Terpenoids)

The extract is mixed with 2ml of chloroform and 2ml of conc. H_2SO_4. And the formation of brown ring at the interface confirms the presence of steroids or terpenoids [82].

3.9 Ferric Chloride Test

The extract is taken in a test tube, 2-3 drops of 5%$FeCl_3$ solution is added. The formation of blue-black, green or violet colour indicates the presence of phenolic compounds [83].

Table 4. phytochemical present in which part of plant

Phytochemical	*Clitoria ternatea part is present*	*Curcuma caesia*
Alkaloids	Root, seed, leaf	Rhizome
Flavonoids	Leaf, flower, seed	Rhizome, leaf
Tannins	Root, seed	Rhizome

Saponins	Seed, leaf	Rhizome
Terpenoids	Root, leaf	Rhizome
Glycosides	Leaf, flower, seed	Rhizome
Steroids	Leaf, seed	Rhizome
Phenolic compounds	Leaf, flower	Rhizome, leaf

4 PHARMACOGNOSTICAL EVALUATION

Pharmacognostic evaluation includes both qualitative macroscopic/microscopic analysis and quantitative physicochemical parameters. These analyses ensure the authenticity, purity, and quality of plant materials [84].

4.1 Qualitative Analysis

1. **Ash value:** Approximately 5 g of the powdered drug is precisely weighed and taken individually in a silica crucible. Then a thin layer of powder was applied to the crucible's bottom. And the powder was burned by progressively increasing the temperature until it became dull red hot and carbon-free. After cooling, the crucible was weighed and to get this process was repeated until the occurrence of constant weight. Then air-dried powder was used as a reference to calculate the percentage of total ash [85].

$$\% \text{ ash content} = \frac{\text{weight of crucible+ash−wt of crucible}}{\textit{weight of crucible+sample−wt of crucible}} \times 100$$

2. **Moisture content:** take a weighing of the sample is placed into a weighing bottle that has been accurately weighed. Then it is dried at 105ºC for 5 - 6 hours and then it is cooled in desiccators with silica gel. And when the material is dried to a constant weight, the moisture content is determined and calculated [86].

$$\text{Moisture content} = \frac{\text{weight of petridish+crude drug−after drying wt of petridish+sample}}{\textit{weight of crude drug}} \times 100$$

3. Determination of extractive value (Alcohol)

Extractive value (Alcohol): A 250 mL conical flask with a stopper was taken and 100 mL of 90% ethanol was weighed with the powdered substance, and the stopper was then replaced. And with the help of mechanical shaker shaken for 6 hours, after that the flask and its contents were left to stand for 18 hours. After the filtration of mixture, the filtrate were measured and evaporated until dry in an evaporating dish with a known weight. After drying for approximately three minutes at 105^0C in the oven, the residue's constant weight was obtained. And then the extractive value was calculated [87].

$$\text{Alcoholic soluble extractive value} = \frac{\textit{weight of residue}}{\textit{weight of the drug}} \times 100$$

4.2 Quantitative Analysis

1. Total Flavonoid Content (TFC)

Clitoria ternatea

Total flavonoids were estimated following the method of Luximon-Ramma et al. (2002). Five hundred milligrams of dry plant powder (leaves, stem, root, seed, and flower) was extracted with 10 mL of 80% acetone using a mortar and pestle. The

homogenate was filtered through a Buchner funnel with Whatman No. 1 filter paper, and the filtrate was adjusted to 50 mL with 80% acetone.

For the assay, the plant extract was weighed and mixed with 1.5 mL of 2% methanolic aluminium chloride solution (2 g $AlCl_3$ dissolved in 100 mL methanol). A blank was prepared by replacing the extract with distilled water. The absorbance of the reaction mixture was reported at 368 nm using a UV-Visible double beam spectrophotometer. Total flavonoid content was calculated using a standard curve of rutin. [88].

Curcuma caesia

The total flavonoid content was measured using the Aluminium Chloride ($AlCl_3$) method, with quercetin as the standard. Flavonoid levels were expressed in terms of quercetin equivalents. A calibration curve was prepared using quercetin solutions at concentrations of 20, 40, 60, 80, and 100 mg/L.

For the assay, the standard or extract was placed in a 10 mL volumetric flask containing 4 mL of distilled water. Then, 0.3 mL of 5% sodium nitrite ($NaNO_2$) was added. After 5 minutes, 0.3 mL of 10% aluminium chloride ($AlCl_3$) was introduced, followed by 2 mL of 1 M sodium hydroxide (NaOH) at the 6th minute. The final volume was made up to 10 mL with distilled water, and the absorbance was reported using a UV-Visible spectrophotometer [89].

2. **Antioxidant Activity**

Clitoria ternatea

The antioxidant activity of *Clitoria ternatea* extracts was measured using the DPPH assay, and it was found that the aqueous extract had stronger activity than the ethanol extract. This activity is mainly due to phenolic compounds present in the plant. At lower concentrations, the aqueous extract showed moderate inhibition of DPPH radicals [90].

Curcuma caesia

For Curcuma caesia antioxidant activity same procedure was followed. Test solutions were prepared at concentrations of 0, 237.6, 475.2, 712.8, and 950.4 ppm to create a calibration curve relating concentration to absorbance [91].

The maximum absorbance of DPPH was reported at 517 nm, where the radical exhibits strong absorption. Measurements showed that higher concentrations of black turmeric extract reduced absorbance, indicating stronger free radical scavenging activity [92].

3. Antimicrobial activity

Clitoria ternatea

The antibacterial activity of *Clitoria ternatea* crude extracts was evaluated using a modified disk-diffusion method [40,41].

The discs were carefully placed on Mueller-Hinton Agar (MHA) plates inoculated with *Escherichia coli*. The bacterial inoculum was standardized to 0.5 McFarland standard (~1.5×10^8 CFU/mL) and spread over the agar surface using a sterile cotton swab. Plates were left at room temperature for 15 minutes to allow absorption and 1 hour before incubation to facilitate extract diffusion. Plates were incubated at 37°C for 24 hours.

After incubation, the zones of inhibition around each disc were measured in millimeters using a ruler. All experiments were performed in triplicate, and results were expressed as mean ± standard deviation [93].

Curcuma caesia

Sterile swabs were used to inoculate Muller-Hinton Agar (MHA) plates with the test organism, and the plates were allowed to absorb for 10 minutes. Using a sterile well borer, four wells were created in each agar plate. The plant extract was taken at different concentrations (200 mg/mL, 100 mg/mL, and 50 mg/mL) and DMSO as a negative control were added to the respective wells. The plates were left at room temperature for 1 hour to allow absorption of the extract, then incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured and recorded [94].

4. Discussion

Medicinal plants have been used for centuries in traditional medicine due to their rich content of bioactive compounds, which provide various health benefits and serve as a primary source of nutrients [1]. In modern times, there is growing interest in plant-based medicines because of their global acceptance, safety, and affordability, positioning them as promising candidates for drug discovery and therapeutic applications [2]. *Clitoria ternatea* (Butterfly Pea) is an herbaceous climber known for its neuroprotective, memory-enhancing, antidepressant, and antioxidant effects. These pharmacological activities are largely attributed to its bioactive compounds, such as anthocyanins, flavonoids, and triterpenoids, which also impart the characteristic blue color of the flowers [3]. Similarly, *Curcuma caesia* (Black Turmeric) contains alkaloids, terpenoids, flavonoids, tannins, and essential oils such as camphor, ar-turmerone, and borneol. These compounds have been associated with antimicrobial, antioxidant, anticancer, and anti-inflammatory effects, validating its traditional use in treating respiratory infections, wounds, and fever [4]. The skin acts as a physical barrier protecting the body from environmental threats, and plant-based compounds have long been used in topical applications for dermatological purposes [5]. Phytochemicals such as flavonoids, phenolics, saponins, and terpenoids contribute to antioxidant and antimicrobial activity, highlighting the relevance of *Clitoria ternatea* and *Curcuma caesia* in skin-related formulations [6]. The method of extraction and choice of solvent play a critical role in determining the yield and efficacy of bioactive compounds. Ethanolic extracts of *Curcuma caesia* rhizomes show significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, emphasizing the importance of extraction conditions in maximizing therapeutic potential [7]. Both plants can provide concentrated bioactive metabolites when appropriate extraction methods are used. *Curcuma caesia* contains various phytochemicals, including alkaloids, terpenes, amino acids, carbohydrates, tannins, flavonoids, steroids, proteins, and other secondary metabolites such as camphor, ar-turmerone, borneol, and ocimene. These constituents support its traditional use in treating pneumonia, cough, fever, wounds, and snake or scorpion bites, as well as providing antioxidant and antimicrobial effects [8]. In contrast, *Clitoria ternatea* contains anthocyanins, triterpenoids, flavanol glycosides, and steroids, which are responsible for its use in treating body aches, infections, urinogenital disorders, and as an antidote to animal stings [9]. The pharmacological activities of both plants are strongly linked to their phytochemical composition. Tannins, terpenoids, flavonoids, alkaloids, phenols, and saponins from *Curcuma caesia* exhibit anti-inflammatory, antimicrobial, antioxidant, and anticancer activities [10]. Likewise, *Clitoria ternatea* contains saponins, carbohydrates, triterpenoids, phenols, steroids, and anthocyanins, which contribute to antioxidant, antidiabetic, antimicrobial, anti-inflammatory, and antipyretic effects [11]. Ethanolic extracts of *Clitoria ternatea* show broad-spectrum antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*, with leaves and roots demonstrating the highest potency [12]. Essential oils extracted from *Curcuma caesia* rhizomes have also shown activity against Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Salmonella typhimurium*), validating their antimicrobial potential [13]. The antioxidant properties of these

plants have been evaluated using DPPH assays. Among *Curcuma caesia* extracts, ethanol shows the strongest free radical scavenging activity, while methanol, ethyl acetate, and aqueous extracts also exhibit moderate effects [14]. Similarly, aqueous extracts of *Clitoria ternatea* show higher antioxidant activity than ethanolic extracts, with leaf and stem extracts being more effective than seeds [15]. These results correlate with the phenolic and flavonoid content, suggesting their critical role in combating oxidative stress. Anthelmintic activity has been observed in both plants, with earthworm models demonstrating paralysis and death upon exposure to plant extracts [16]. Furthermore, anti-asthmatic activity has been documented for *Curcuma caesia*, with methanolic extracts showing bronchodilatory effects in histamine-induced guinea pig models, outperforming standard drugs in some aspects [17]. *Clitoria ternatea* also provides vision protection due to its antioxidant properties, which help mitigate free radical damage in ocular tissues and prevent disorders such as glaucoma and retinal damage [18]. Additionally, *Curcuma caesia* demonstrates anti-inflammatory activity by inhibiting protein denaturation and cyclooxygenase activity, as measured by standard assays [19]. The antidiabetic potential of both plants is linked to their ability to inhibit carbohydrate-hydrolyzing enzymes such as alpha-amylase. Methanol extracts of *Curcuma caesia* exhibit strong alpha-amylase inhibitory activity, while n-hexane and water-soluble residues show moderate effects [20]. Cytotoxic and anticancer effects have been reported for *Clitoria ternatea*, where petroleum ether and ethanolic extracts induce dose-dependent reductions in cell viability [21]. Phytochemical screening confirms the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, steroids, and phenolic compounds in both plants, distributed across different plant parts. *Curcuma caesia* mainly contains these compounds in rhizomes and leaves, while *Clitoria ternatea* exhibits them in flowers, leaves, roots, and seeds [78–83]. Pharmacognostic evaluation and quantitative analysis, including total flavonoid content and antioxidant assays, support the therapeutic potential of these plants. Extractive values, ash content, and moisture content provide additional measures of quality and standardization [88–92, 93, 94]. Overall, *Curcuma caesia* and *Clitoria ternatea* are promising candidates for drug discovery and formulation development, particularly in dermatological applications, polyherbal therapeutics, and functional foods. Their complementary pharmacological profiles suggest potential synergistic benefits, and further research focusing on bioactive isolation, mechanism elucidation, and clinical validation is warranted.

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