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EXTRACTION, PHYTOCHEMICAL INVESTIGATION AND *IN VITRO* ANTIMICROBIAL OF EXTRACT OF *CURCUMA CAESIA*

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ABSTRACT

Curcuma caesia, commonly known as black turmeric, is a perennial herbaceous plant native to the Indian subcontinent. It is renowned for its dark purple to black rhizomes and has been traditionally used in Ayurvedic and traditional Chinese medicine for its medicinal properties. This review summarizes the extraction methods, phytochemical constituents, and in vitro antimicrobial activities of *Curcuma caesia* extract. The aqueous extraction yielded 7.56%, and phytochemical screening revealed the presence of alkaloids, glycosides, diterpenes, phenols, flavonoids, and proteins. The extract exhibited significant antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, with zones of inhibition ranging from 11 to 14 mm at 100 mg/ml concentration. These findings highlight *Curcuma caesia* as a potential source of natural antimicrobial agents, underscoring its pharmacological potential and suggesting avenues for further research.

Key Words: *Curcuma caesia*, black turmeric, phytochemicals, antimicrobial activity, extraction methods

INTRODUCTION

Curcuma caesia, commonly known as "black turmeric," is a perennial herbaceous plant belonging to the ginger family, Zingiberaceae. It is native to the Indian subcontinent and widely distributed in regions of Northeast India, particularly in Manipur, and other parts of Southeast Asia (Agarwal *et al.,* 2013; Prakash *et al.,* 2014). The plant is renowned for its distinct dark purple to almost black rhizomes, which set it apart from other turmeric species known for their bright yellow-orange coloration.

Throughout history, *Curcuma caesia* has been valued in traditional medicine systems such as Ayurveda and traditional Chinese medicine for its purported medicinal properties (Sharma *et al.,* 2017; Singh *et al.,* 2016). Various parts of the plant, particularly the rhizomes and roots, are known to contain a diverse array of bioactive compounds that exhibit pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties.

Phytochemical investigations have identified several key compounds in *Curcuma caesia*, notably curcuminoids such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin, which are responsible for its pharmacological effects (Agarwal *et al.,* 2013; Prakash *et al.,* 2014). These compounds have been extensively studied for their antioxidant properties, which contribute to their potential in combating oxidative stress-related diseases.

The antimicrobial activity of *Curcuma caesia* extracts has also garnered significant interest. Studies have demonstrated its effectiveness

against a wide range of pathogenic microorganisms, including bacteria, fungi, and even some viruses (Sharma *et al.,* 2017; Singh *et al.,* 2016). This antimicrobial activity is attributed to the presence of bioactive compounds that disrupt microbial cell membranes, inhibit enzymatic processes, or interfere with microbial DNA replication.

Despite the growing body of evidence supporting its medicinal benefits, there remains a need for comprehensive studies focusing on the extraction methods, phytochemical composition, and detailed evaluation of its antimicrobial potential through robust in vitro experiments. Such investigations are essential not only for validating its traditional uses but also for exploring its therapeutic applications in modern medicine.

This paper aims to review and present findings from research studies on Curcuma caesia, emphasizing its extraction methods, phytochemical constituents, and in vitro antimicrobial activities. By synthesizing existing knowledge and addressing gaps in research, this study contributes to the broader understanding of *Curcuma caesia's* medicinal properties and its potential as a source of novel antimicrobial agents.

MATERIALS AND METHODS

Collection of *Curcuma caesia*

Fresh rhizomes of *Curcuma caesia* were collected from Local areas of Bhopal on the basis of geographical availability. The collected parts of *Curcuma caesia* were authenticated at the Botanical Survey of India, Bhopal. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Drying and Storage

Rhizomes were washed and dried under shade. After drying, they were ground to fine powder for further analysis.

Powdering

After drying, the plant materials were grind well using mechanical blender into fine powder and transferred into airtight containers with proper labeling for future use.

Extraction of *Curcuma caesia* using maceration

The extract was prepared by maceration method. Fresh black turmeric rhizomes collected, shade-dried, and made into powder. 30 gram powdered were maceration with aqueous solvent (v/v) with proper namely rhizomes of *Curcuma caesia* and packed in extraction bottle for 24 hours. The extract was filtered first using muslin cloth and then with Whatman filter paper No. 1. Excess of solvent was evaporated by rotary evaporator at 40°C to get semisolid texture of extract. The extract obtained with solvent was weighed on a constant weight and calculated on the basis of percentage w/w. Finally powdered extracts was weighed and transferred in clean and dried vial, then stored in refrigerator at 4°C until use (Nurhadi *et al.,* 2020).

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula (Nkafamiya *et al.,* 2010):

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical screening

Medicinal plants are traditional pharmaceutical commodities and many of the current medicinal drugs are derived indirectly from plants.

Phytochemical materials consist of two main bioactive components (chlorophyll, vitamins, amino acids, sugar etc.) and secondary bioactive components; (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods (Doss *et al.,* 2009).

1. **Detection of alkaloids:** Extract were dissolved individually in dilute Hydrochloric acid and filtered.

Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates was treated with Hager's reagent (saturated picric acid solution).Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Benedict's Test: Filtrates was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's (combined reducing sugars) Test: Filtrates was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extract was treated with dil. H_2SO_4, formation of red color solution indicate the presence of glycosides.

4. Detection of saponins

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

***In-vitro* antimicrobial activity**

The reference microbial species; Microbial Type Culture Collection (MTCC) of *Staphylococcus aureus* and *Escherichia coli* was collected from Bacteriology Unit of the Microbiology Laboratory. The 24hrs old bacterial strains were prepared by inoculating

a loopful of original cultures in nutrients broth and incubate overnight at 37^0C.

Table 1: Composition of nutrient agar for bacteria

S. No	Ingredients	Gram/liter
1	Peptone	5.0
2	Yeast extract	1.5
3	Beef extract	1.5
4	Sodium chloride	5.0
5	Agar	20.0
Final pH at 25°C: 7.4 ±0.2		

Media preparation and sterilization

Agar well diffusion method was performed for screening of antibacterial activity. Nutrient agar media (NAM) was prepared for growing of bacteria inside the laboratory. The standard size (100mm× 15mm) petri dishes as required for whole experiment. For preparation of NAM (Table 1), ingredients was mixed with 1000 ml of distilled water and stirred to obtain homogenized mixture. After which, NAM mixture were placed in Autoclave under 15 psi pressure, at 121°C for 25 min for sterilization of media. The media was prepare and poured at a rate of 18 ml each in a petri dish and allowing for solidification for about 15 min. A loopful of inoculums was swabbed uniformly onto the media and left them drying. The different concentration of extract of (100 mg/ml, 50 mg/ml and 25 mg/ml) was loaded into each well with the help of 6 mm agar well cutter and allowed diffusion of extract. Plates are incubated overnight at 37°C zone appeared after incubation period and the percent of incubation was determined in millimetres using scale (Parekh *et al.,* 2007).

RESULTS AND DISCUSSION

The aqueous extract of *Curcuma caesia* yielded 7.56%, indicating the efficiency of the extraction process in obtaining bioactive compounds from the plant material. Phytochemical screening revealed the presence of various phytoconstituents. Alkaloids, glycosides, diterpenes, phenols, flavonoids, and proteins were detected in the aqueous extract, while saponins, carbohydrates, and tannins were absent or present in minimal quantities. These findings suggest that Curcuma caesia contains a diverse array of phytochemicals that contribute to its medicinal properties, including antioxidant, antimicrobial, and potentially other pharmacological activities.

The antimicrobial assay demonstrated significant activity of the *Curcuma caesia* extract against both *Staphylococcus aureus* and *Escherichia coli*. At concentrations of 100 mg/ml, the extract exhibited notable zones of inhibition: 14 ± 0.86 mm for *Staphylococcus aureus* and 11 ± 0.94 mm for *Escherichia coli*. These results indicate the extract's potential as a natural antimicrobial agent, which could be attributed to the presence of bioactive compounds like alkaloids, phenols, and flavonoids identified in the phytochemical screening.

The findings underscore the pharmacological potential of *Curcuma caesia* as evidenced by its extractive values, phytochemical profile, and antimicrobial activity. The high yield of aqueous extraction coupled with the presence of diverse phytoconstituents suggests that *Curcuma caesia* could be further explored for its therapeutic benefits in treating microbial infections and other health conditions. The antimicrobial results are particularly

promising, indicating that the extract may offer an effective alternative or complementary treatment to conventional antibiotics.

The comprehensive analysis of *Curcuma caesia* extract highlights its rich phytochemical composition and potent antimicrobial properties, laying the foundation for its continued exploration and development as a natural therapeutic agent.

Table 2: Extractive values obtained from *Curcuma caesia*

S. No.	Extract	Color of extract	% Yield
1	Aqueous	Orange- brown	7.56%

Table 3: Preliminary phytochemical screening of *Curcuma caesia* extract

S. No.	Phytoconstituents	Aqueous extract
1.	Alkaloids	
	Wagner's Test	+
	Hager's Test	+
2.	Glycosides	
	Legal's test	+
3.	Saponins	
	Foam test	-
4.	Diterpenes	
	Copper acetate test	+
5.	Phenols	
	Ferric chloride test	+
	Folin–Ciocalteu	+
6.	Carbohydrates	
	Fehling test	-
	Benedicts test	-
7.	Flavonoids	
	Lead acetate	+
	Alkaline reagent test	-
8.	Proteins	
	Xanthoproteic test	+
9.	Tannin	
	Gelatin test	-
10.	Sterol	
	Salkowski test	-

(+)=positive; (-) = negative

Table 4: Result of antimicrobial assay of *Curcuma caesia* extract

Microbes	Zone of Inhibition (mm)		
	25mg/ml	50 mg/ml	100 mg/ml
Staphylococcus aureus	10 ± 0.47	11± 0.5	14 ± 0.86
Escherichia coli	7±0.57	9±0.74	11±0.94

N=3, Average of three value

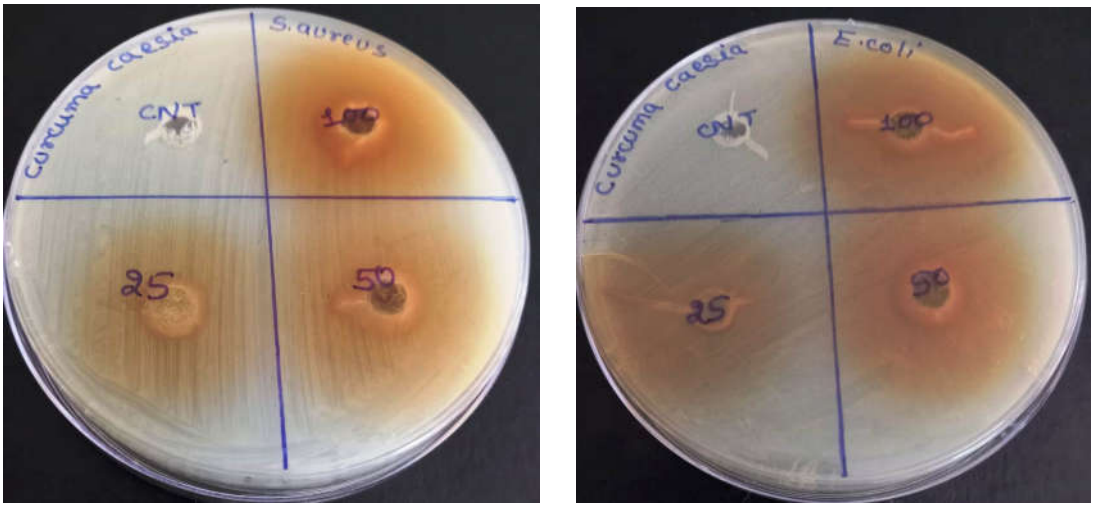

Figure 1: Photoplates of antimicrobial assay of plant extract

CONCLUSION

In conclusion, *Curcuma caesia* extract emerges as a promising candidate in the search for natural antimicrobial agents, offering potential benefits for both traditional and modern medicinal applications. Continued research and development efforts are essential to fully harness its therapeutic potential and pave the way for its integration into healthcare practices.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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