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Preliminary-phytochemical screening and antimicrobial activity of *Curcuma caesia*

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ABSTRACT

Chhattisgarh State of India is known for its high diversity of medicinal plants. A comparative investigation was done on the phytochemical profile and bioactive properties of methanolic extracts obtained from leaves and rhizomes of *Curcuma caesia* collected from Chhattisgarh, India. Different levels of flavonoids, tannins, alkaloids, steroids, and terpenoids were confirmed by qualitative assay. Results of quantitative analysis showed that the leaf extract had higher total flavonoid content (13.76 mg QE/g) and the rhizome extract had higher total phenolic content (13.329 mg GAE/g). Presence of high TPC and TFC indicate the antioxidant property of *C. caesia.* The rhizome extract shows moderate to strong antimicrobial activity against gram positive bacterial strains such as *Bacillus safensis*, *Bacillus pumilus, Staphylococcus aureus,* and gram-negative bacteria *Escherichia coli*. The value of zone of inhibition shows the high antimicrobial activity against both gram +ve and -ve bacteria. This study shows that *Curcuma cassia* contains many phytochemical components that can be used in many pharmaceutical industries in future.

Figures : 04 References : 24 Tables : 03
KEY WORDS : Antimicrobial activity, *Curcuma caesia,* Phytochemical, Secondary metabolites, TPC, TFC.

Introduction

Curcuma caesia commonly known as black turmeric, has very high medicinal value and it is one of the endangered species found distributed in forest of Indian Subcontinent[16-18,21]. Black turmeric is a rhizomatous herb that grows erect and belongs to family Zingiberaceae, with the composition of bluish-black rhizome. It possess a strong camphoraceous smell and has been utilized in pharmaceutical industries[20]. It is already established that plants have a wide-pharmacological properties that resulting from the existence of secondary metabolites[6,17]. These secondary metabolites have significant pharmacological potential such as isothiocyanates and sulfur compounds, known for antioxidant and anticancer potential[1,4,5]. The medicinal value of *C. caesia* has largely concentrated on its rhizome and leaf and their phytochemical and therapeutic potential is fully underexplored. The Adi tribe from Arunachal, India, used a decoction made from the fresh rhizomes of this plant to treat stomach-aches[6,8]. Further, the aim of this study is to analyse the comparative assessment of phytochemical constituents of leaf and rhizome extracts to fulfil the research gap. This study represents the first attempt to investigate the phytochemical, antimicrobial activity, TPC, TFC and MIC determination of *C. caesia* collected from Chhattisgarh, which will provide valuable information for the local healer and baigas of Chhattisgarh, those are practiced in traditional medicinal system.

Materials and Methods

Study area

Healthy (showing no visual disease symptoms) and mature plants were collected from the medicinal

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TABLE-1: Phytochemical screening of *Curcuma caesia* from leaves and rhizome

S. No.	Test	Reagents	Result	
			Leaf	Rhizome
1.	Carbohydrate	Molish's test	+	+
2.	Reducing Sugar	Fehling's solution	++	+
3.	Flavonoids	Lead acetate test	++	+++
		Alkaline reagent test	-	++
4.	Tannins	Lead acetate test	++	++
		Ferric chloride	++	++
5.	Amino acids	Ninhydrin	-	-
6.	Alkaloids	Mayer's test	+	+
		Wagner' s test	++	+
7.	Tannin	Bromine water	+	++
8.	Steroid	Salkowaski	+	+
9.	Terpenoids	Salkowski Test	-	++
10.	Fat and oils	Solubility test	-	-

*+++ = Strongly present; ++ = moderately present; + = present; - = absent

plant garden of the Department of Rural Technology and Social Development. Campus Guru Ghasidas Vishwavidyalaya (a central university) which is located in Koni village of Bilaspur, Chhattisgarh, India.

Collection and Identification

The sample was collected in January 2021 and an herbarium of plant was prepared. The whole plant, including rhizome and leaves were collected for the phytochemical screening. The plant was identified on the basis of morphology (Fig. 1 A & B). The plant identification cell of the Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh (Bot/GGV/2022/47) confirm the identity of plant species as *Curcuma caesia*.

Preparation of Methanolic Extracts (Soxhlet Method)

The fresh leaves and rhizome of *C. caesia* were thoroughly washed with distilled water 2-3 times, shade-dried for 7-10 days after making small pieces (Fig. 1 C &D), and then ground into a coarse powder (2-3mm) using a mortar pestle[15]. Soxhlet apparatus was used to prepare methanolic extract of 5g pre-soaked sample of leaf and rhizome. The extracts were kept for evaporation of solvent and then stored in sterile airtight vessel at 4°C for further phytochemical and anti-microbial activity[21].

TABLE-2 : Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in Plant extract.

S. No	Plant Sample	Extraction Yield	TPC (mg GAE/g)	TFC (mg QE/g)
1.	Rhizome	47.4	13.329 ± 0.010	11.282±0.0697
2.	Leaf	27.4	13.168 ± 0.263	13.765 ± 0.1638

Extraction Yield

To compare the extraction yield of plant parts, the yield was determined by using following formula:

$$\text{Extraction Yield} = \frac{\text{Weight of dried extract}}{\text{Initial weight of sample}} \text{ X100}$$

Qualitative analysis

Standard procedures were employed to conduct a phytochemical screening of the crude extracts, aiming to detect flavonoids, tannins, amino acids, steroids, sugars, terpenoids, and alkaloids[8,12,23].

Quantitative analysis

Total Phenolic Content (TPC)

Total Phenolic Content from crude extract of leaf and rhizome was estimated by using Folin-Ciocalteau's reagent (10%, v/v) and sodium carbonate solution (7.5%) measured at 765 nm using a UV-Vis Double Beam Spectrophotometer (Systronics). A similar procedure was performed for variable concentrations of Gallic acid (5-100µg/ml). The total phenol content was subsequently calculated using a Gallic acid standard curve. The results were expressed as milligram Gallic acid equivalents (GAE)/g of sample [2,3,19,23].

Total Flavonoid Content (TFC)

The methanolic extracts leaf and rhizome were determined by dissolving 1mg of extract with 1 ml of methanol. Then 0.5 ml of 1mg/ml sample was mixed with 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1ml. potassium acetate, and 2.8 ml of distilled water. This mixture was incubated at room temperature for 30 min. The absorbance was measured at 415nm using a UV-Vis Double Beam Spectrophotometer (Systronics). A similar treatment was performed for variable concentrations of Quercetin (5-100ìg/ml). The TFC was calculated using Quercetin as a standard, and values were expressed in terms of Quercetin equivalents (mg QE)/g of the sample[24].

Antimicrobial Activity of Leaf and Rhizome Extract

The disc diffusion assay was performed to evaluate the antimicrobial activity of leaf and rhizome extracts against selected bacterial strains. The bacterial strains, including *Bacillus safensis* (F1), *Bacillus pumilus* (P1), *Staphylococcus aureus* (WR3), and *Escherichia coli* (DH5-á), were cultured in nutrient broth and incubated at optimal growth conditions. Mueller-Hinton agar plates were used for antimicrobial assay. Sterile filter paper discs (6mm) were impregnated with the test compounds at predefined concentrations and carefully inoculated on agar surface using sterile forceps. Discs containing standard antibiotics and solvent controls were also included for comparison[4,9,14]. The size of the inhibition zone was recorded (after 24 hours) for each test sample, and results were analysed to determine the relative antimicrobial efficacy of the leaf and rhizome extracts[14].

Minimum Inhibitory Concentration (MIC) Determination

The Minimum Inhibitory Concentration (MIC) of plant extract was determined using a microdilution assay following the Clinical and laboratory Standards Institute

TABLE-3 : Average Minimum Inhibitory Concentration (MIC) of *C. caesia* crude extract of Rhizome and leaf.

Sample Name	Sample Pair	Average MIC Well	Average MIC Concentration (µg/mL)
Rhizome	C & D	7	0.0469
Leaf	G & H	7.5	0.0332

Fig.1: *Curcuma caesia* (a) Whole plant, (b) Rhizomes, (c) Leaf Samples and (d) Rhizome samples ready for extraction

(CLSI) protocols. A U-shaped 96-well microtiter plate was used for the MIC assay. Each well contained 100 µL of serially diluted bee venom extract in Potato Dextrose Broth. Plant extracts were dissolved in distilled water at a concentration of 2mg/mL and filtered using a 0.22µm pore-size Rotilabo® syringe filter to ensure sterility. The filtered extract was serially diluted in Potato Dextrose broth (PDB) to obtain a range of concentrations for testing. Potato Dextrose broth supplemented with 10% dimethyl sulfoxide (DMSO) has been used a positive control to ensure that the solvent had no antimicrobial effects. Additionally, an extract control containing Potato Dextrose Broth with 10% DMSO and bee venom extract was used to confirm the antimicrobial activity of the sample. The plates were incubated at 25°C for 24 hours to allow fungal growth[7].

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± Standard Deviation. All the data were recorded and analysed with the help of MS Excel for the calculation.

Results and Discussion

The preliminary phytochemical screening of *C. caesia* leaves and rhizomes was done and the absence or presence of plant metabolites was recorded on the basis of intensity of colour of solution performed for various test with selective reagents. Results show that rhizome has a wider variety of phytochemicals than the

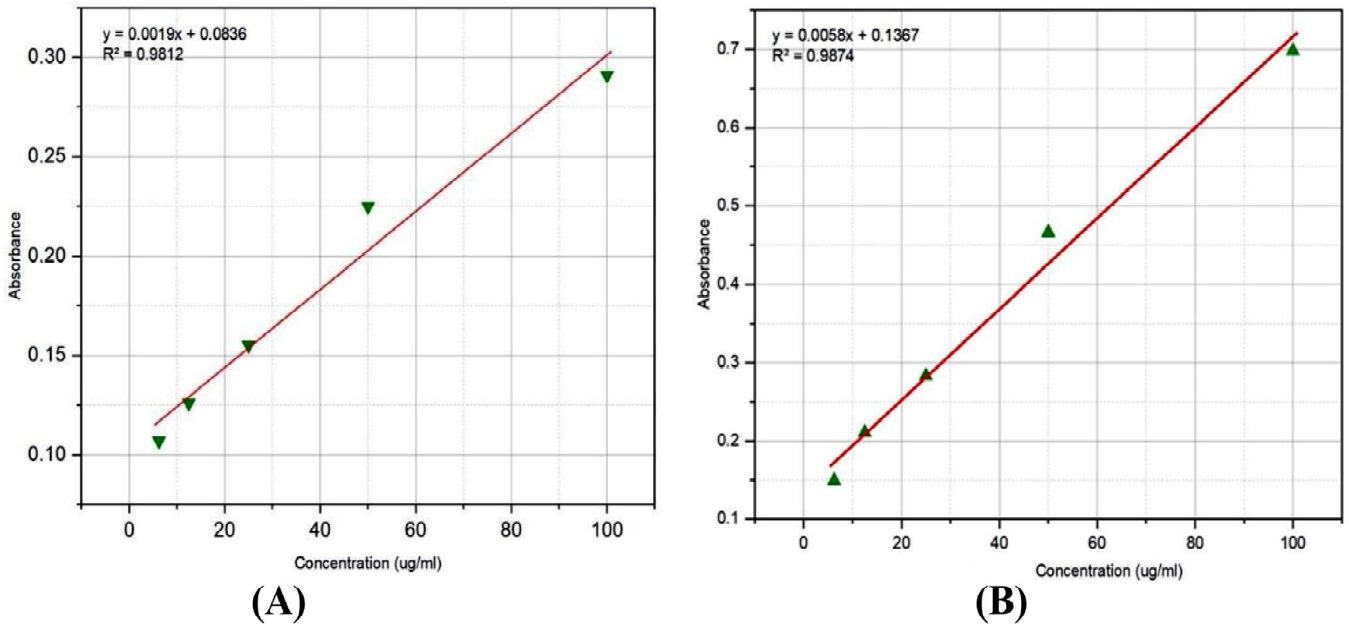

Fig. 2: Standard curve for (A) TPC (Gallic acid) and (B) TFC (Quercetin).

leaf (Table-1). Previous studies also reported the richness of phytochemical in rhizome[11]. Flavonoids (Alkaline reagent test), Terpenoids (Salkowski Test) in leaf extract and Amino acid (ninhydrin test) and Fat and oils (solubility test) was not detected in both leaf extract and rhizome extract. These results were also in agreement with the earlier reports[8,11, 16,17].

Plant contained phenolic and flavonoid compounds in their different parts. These phenolic and flavonoid compounds are attributed to antioxidant activity, free radical-scavenging activity and metal chelating properties. In view of the importance of phenolic and flavonoid compounds, Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were investigated in leaves and rhizomes of *C. caesia* plants. The data showed that the plant rhizome had higher TPC than the

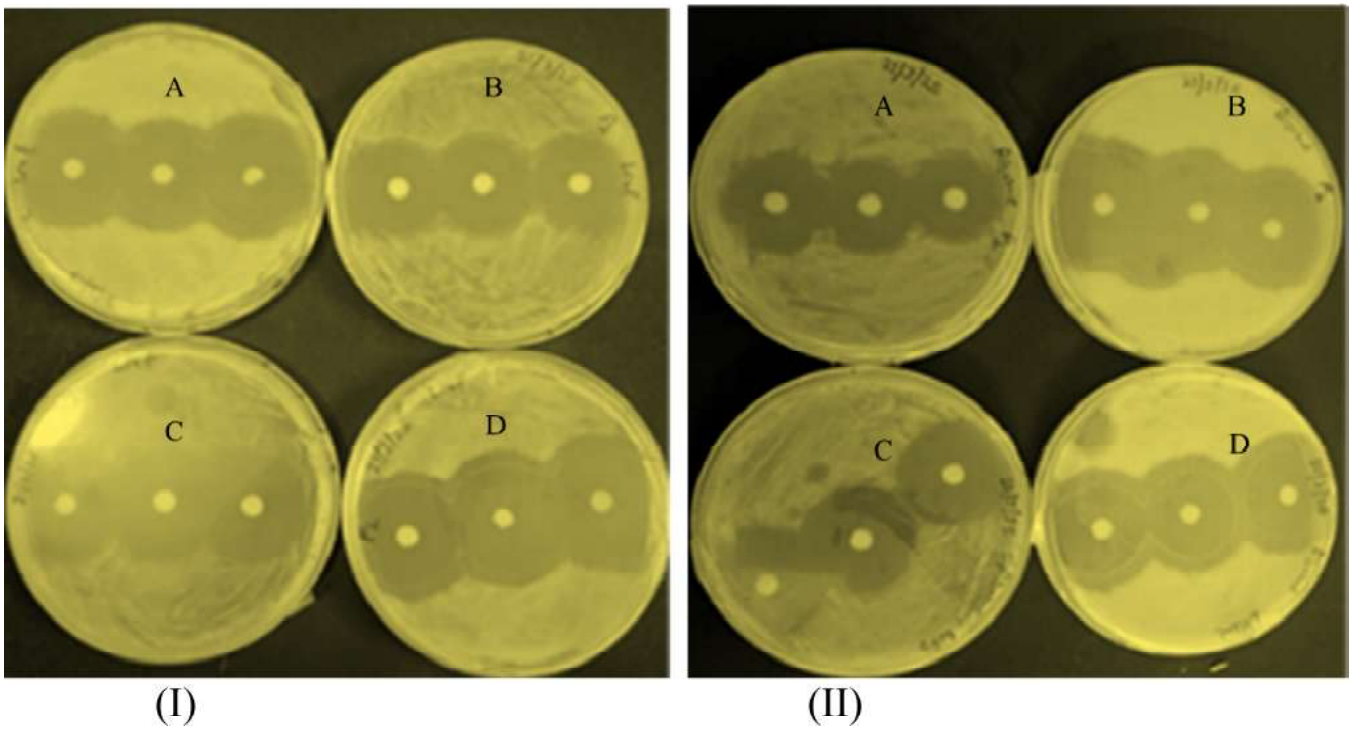

Fig. 3: Anti-microbial activity of leaf extract (I) and Rhizome of *C. caesia* (II) against (A) *Bacillus pumlius*, (B) *Bacillus safensis*, (C) *Escherichia coli*, (D) *Staphylococcus aureus*.

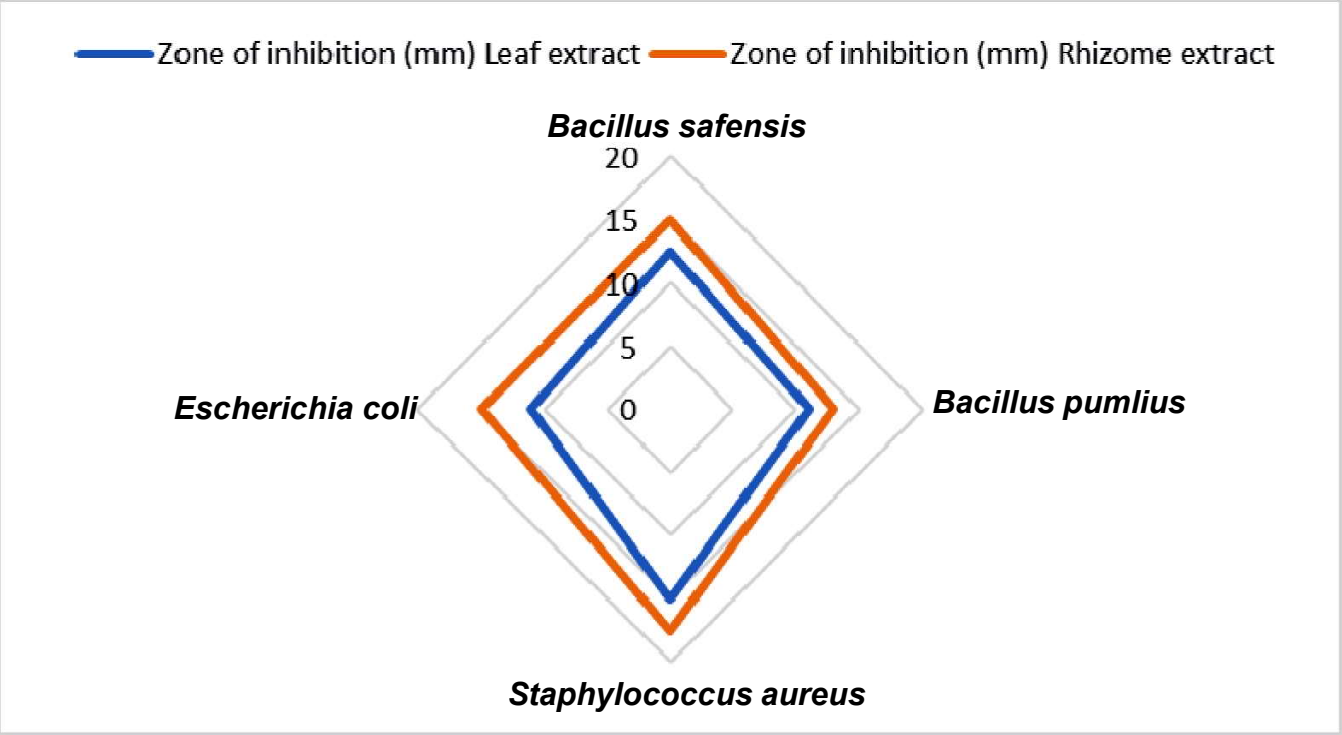

Fig. 4: Zone of inhibition and antimicrobial activity level of leaf and Rhizome Extract

leaf. In contrast, the plant leaf had higher TFC than the rhizome. It can be concluded that leaves of *C. caesia* plants have higher TFC levels, whereas rhizomes have higher TPC levels (Table-2, Fig.2). This result is supported by the findings of previous studies[11,19,22]. This similarity with previous finding confirms the phytochemical richness in leaf and rhizome of *C. caesia* found naturally in Chhattisgarh[20].

The present study successfully established a comparative phytochemical profile of *C. caesia* leaf and rhizome extracts using both qualitative and quantitative analyses. The findings revealed significant levels of total phenolic content (TPC) and total flavonoid content (TFC), with values comparable to those previously reported in literature [18].

Minimum Inhibitory Concentration (MIC)

.The MIC assay revealed differences in the susceptibility of fungal strains from Rhizome and Leaf samples. The MIC value for the rhizome sample was recorded at 0.0469 µg/mL, whereas. The Leaf sample exhibited a lower MIC value of 0.0332 µg/ml (Table-3). The results were consistent across triplicate experiments, confirming the reproducibility and reliability of the MIC values obtained [13].

Antimicrobial activity

To effectively combat microbial infections, it is essential to understand the antimicrobial property of plant. The Disc Diffusion Assay was used to determine the antimicrobial property of crude extracts of leaf and rhizome (Plate 2 I-II). Antimicrobial activity was tested against three gram-positive *i.e. Bacillus safensis, Bacillus pumlius, Staphylococcus aureus,* and one gram-negative *Escherichia coli* bacteria (Fig. 3). The crude extracts of leaf and rhizome both show the antimicrobial property against selected bacteria. The crude extract of rhizome showed the highest inhibition up to 17.5mm against the *S. aureus*, suggesting it has stronger antibacterial properties[19,20] (Fig. 4).

Conclusion

The *Curcuma caesia* is an endangered medicinal plant which is found distributed in the forest of Chhattisgarh. The result of present study shows that the crude extract of *C. caesia* possesses many important phytochemical constituents. TPC and TFC value showed that a good amount of phenolic and flavonoid compounds presents which are responsible for antioxidant property of plant. Data of MIC and anti-microbial activity also indicated that the crude extract of *C. caesia* had inhibitory effect against the gram positive as well as gram negative bacteria. Further exploration and exploitation of medicinal plants is needed to determine their medicinal and other beneficial propertied for the betterment of humankind with sustainable approach.

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