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Evaluation of Phytochemical Constituent, Antioxidant Activity and Anti-Bacterial Activity of Black Turmeric (*Curcuma caesia* Roxb.)

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ABSTRACT

The essential oil obtained from *Curcuma caesia* Roxb. rhizome was used to evaluate the preliminary phytochemical screening, total flavonoid and phenolic contents and to access their antioxidant and antibacterial activity. Phenols, flavonoids, tannins, glycosides and carbohydrates were found to be present whereas saponins and proteins were absent. Antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The highest inhibition of essential oil was up to 79.8% at a concentration of 100µl/ml. The total phenol content was calculated by using standard graph of catechol and the highest phenol content was found to be 74.4 ± 0.06 mg/100g. The total flavonoid content was estimated using the quercetin standard curve. The presence of flavonoid was found to be 3.0±0.031mg/100g. Disc diffusion techniques was used to analyses the antibacterial activity against Gram positive bacteria; *Staphylococcus aureus* (MTCC-6908), *Bacillus cereus* (MTCC-430), and Gram-negative bacteria; *Vibrio cholerae* (MTCC-3906), *Salmonella typhimurium* (MTCC-3224) and *E. coli* (MTCC 723). The essential oil showed highest zone of inhibition against *Vibrio cholerae*. Thus, the present study shows that *Curcuma caesia* Roxb. is rich source of antioxidants, phytochemical compounds and antimicrobial activity.

Keywords *Curcuma caesia* Roxb., Essential oil, Phytochemical, Antioxidant, Antimicrobial.

INTRODUCTION

Plants are a source of natural products with varied medicinal characteristics that are constantly being explored to develop novel drugs. They generate secondary metabolites which are made up of many bioactive compounds. These bioactive compounds impart biological activity against several disease-causing agents (Gad *et al.* 2013). Some of the medicinal and aromatic plants are in danger of going extinct because of uncontrolled trading, over harvesting, climate change, and habitat destruction due to the ever-increasing demand for them. One of them, *Curcuma caesia* Roxb., popularly known as black turmeric, is a plant that has been classified as an endangered species because of its traditional use by humans, slow rate of soil reproduction, and root susceptibility to diseases brought on by parasitic fungi called Pythium species. (Behar *et al.* 2013, Haida *et al.* 2022).

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Fig. 1. *Curcuma caesia* plant (A); transverse cut of rhizomes (B).

Curcuma caesia Roxb., is a rare and endangered plant species belongs to family Zingiberaceae. It is a perennial rhizomatous plant often stands upright and in between 0.5 and 1.0 meters tall. It has a broad oblong and glabrous leaves. A deep to light purple color is visible in the middle region of lamina in each leaf. Flowers have a reddish border and are pale yellow in tone. The most potent part is the rhizome (Fig.1), which is tuberous and has a camphoric essential oil with pleasant scent. Its size and shape can vary from 2 to 6 cm. Sessile, lateral flattened, and covered with warts, adventitious roots, and root scars. The interior part of the rhizome is bluish-black or buff in color, with a circular configuration that is frequently misunderstood as the growth ring (Baghel *et al.* 2013). *Curcuma caesia* is found in north-eastern and central India. *Curcuma caesia* also found in the Papi Hills of East Godavari, West Godavari, and Khammam Districts of Andhra Pradesh (Zaman *et al.* 2013). The rhizomes of *Curcuma caesia* have a significant commercial value because of its putative medicinal properties. In Indian system of medicine, the rhizomes of *Curcuma caesia* Roxb. have been used traditionally for treatment of various ailments and metabolic disorders like leukoderma, snake and scorpion bites, asthma, tumours, piles, bronchitis (Baghel *et al.* 2013, Devi *et al.* 2015). The rhizomes are used to treat smooth muscle relaxation. The extract of *Curcuma caesia* is used to treat asthma, cancer, inflammation, epilepsy, fever, and allergies (Israr *et al.* 2012). The rhizomes are used to treat smooth muscle relaxation (Arulmozhi *et al.* 2006). Fresh rhizome decoction is used as antidiarrhoeic and to get relief from stomach ache (Kagyung *et al.* 2010).

MATERIALS AND METHODS

The experiment was conducted at the Department of Horticulture Aromatic and Medicinal Plants (HAMP), Mizoram University in Aizawl, India during the year 2021 to 2023.

Plant collection

Cucurma caesia Roxb. plants were collected from a village called Old Katang, Arunachal Pradesh, India. Samples were collected in the month of Febraury.

Extraction and isolation of essential oil

The fresh rhizome of *Curcuma caesia* Roxb. was sliced and hydrodistilled by Clevengers apparatus (Clevenger 1928) running it for 6-7 hours and essential oil was collected. The oil was dried over anhydrous sodium sulphate to eliminate moisture traces. Then, it was stored in a dark place.

Phytochemical screening

The presence of bioactive agents was determined using standard phytochemical screening protocols. The tests were identified by visual inspection of color change or precipitate formation after the addition of reagents to the solution.

Test for tannin (Braymer's Test)

The presence of tannin was detected by mixing 2 ml of essential oil with 2-3 drops of 5% ferric chloride ($FeCl_2$) as described earlier (Thangjam *et al*. 2020). The formation of blue color was observed to indicate the presence of tannin.

Test for saponin (Foam Test)

Formation of stable foam on the top of the mixture containing 2 ml of essential oil and 5 ml of distilled water in a test tube following vigorous shaking for about 15 mins was observed to indicate of the presence of saponins (Thangjam *et al.* 2020).

Test for flavonoid (NaOH Test)

For detection of flavonoids, 2ml of test sample was mixed with 2ml of 10% NaOH solution. Appearance of yellow to orange color was observed to indicate the presence of flavonoids (Thangjam *et al.* 2020).

Test for phenols ($FeCl_3$Test)

Presence of phenols was detected by adding few drops of 10% $FeCl_3$ to 2ml of the sample. Appearance of blue or green color indicates presence of phenols (Thangjam *et al.* 2020).

Test for protein (Xanthoproteic Test)

2 ml of essential oil was added into 2ml of HNO_3 and boiled in water bath. Orange color was observed to indicate the presence of protein (Thangjam *et al.* 2020).

Test for carbohydrate (Benedict's Test)

Yellow, green or red precipitate was observed to indicate the presence of carbohydrate when mixing 2 ml of essential oil with 2 ml of Benedict's reagent and boiled in water bath (Thangjam *et al.* 2020).

Test for glycosides (Keller-Kiliani Test)

For the detection of glycosides, 2 ml of extract was mixed with 2 ml of glacial acetic acid containing 2 drops of 2% solution of $FeCl_3$. The mixture was poured into another test tube containing 2 ml of concentrated H_2SO_4. Brown ring at the interphase was observed to indicate the presence of cardiac glycosides (Thangjam *et al.* 2020).

Quantitative phytochemical analysis

Determination of phenol content

The total phenolic content was determined according to Folin Ciocalteau method (Sahu and Saxena 2013). 0.5 ml essential oil from the stock solution was taken in a test tube and mixed with 4.5 ml distilled water. Gallic acid was used as a standard. 5 different concentrations of gallic acid (25, 50, 100, 200 and 400 µg/ml) were taken. Then, 0.5 ml of Folin-Ciocalteu reagent was added and shaken and let to rest for 5 minutes. After 5 minutes, 5 ml of 7% Na_2CO_3 was added and the mixture is allowed to stand for 2 hours in the dark with intermittent shaking. The absorbance was taken at 760 nm in a UV spectrophotometer.

Determination of flavonoid content

To determine the total flavonoid content aluminium chloride method was used as described by (Sahu and Saxena 2013). 0.5ml of essential oil was mixed with 0.5 ml of 2% aluminium chloride in a clean test tube. 5 different aliquots (50, 100, 200, 400 and 800 µg/ml) of standard quercetin were taken. Then, 1ml of 2% of aluminium chloride was added into it. The absorbance was recorded at 510 nm using UV-Visible.

Determination of antioxidant content

DPPH radical scavenging activity: Total antioxidant was determined using DPPH assay (Villaño *et al.* 2007). 5 different concentrations of stock solution (10, 25, 50, 75 and 100 µl/ml) were taken in 5 different test tubes and filled with methanol in each test tube

to make the volume up to 1 ml. After this, 1 ml of the prepared DPPH was taken and added to the solution. Then the mixture was shaken vigorously and was left to incubate in a dark room for 30 minutes. In this method, quercetin was used as a standard. After 30 minutes, the absorbance was measured at 517 nm in a UV-Spectrophotometer. The percentage inhibition (scavenging activity) and IC_{50} were calculated.

$$\%\text{DPPH scavenging} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Antibacterial activity

Antibacterial activity of the essential oil obtained from *Curcuma caesia* Roxb., was determined using the disc diffusion method (Bauer *et al*. 1966). This test is based on the measurement of bacterial growth inhibition under controlled conditions. Two gram-negative and two gram-positive bacteria viz., *Salmonella typhimurium* (MTCC 3224), *Escherichia coli* (MTCC 723), *Bacillus cereus* (MTCC 430) and *Staphylococcus aureus* (MTCC 6908) were used for the antibacterial activity.

RESULTS

Phytochemical screening of *Curcuma caesia* rhizome essential oil

The presence of different medicinally active metabolites from *Curcuma caesia* rhizome sample were tested. The sample was found to contain important phytochemicals that are considered to have high medicinal value. The active phytochemicals such as tannins, flavonoids, phenols, glycosides were present in the sample (Table 1).

Table 1. Phytochemical screening of *Curcuma caesia* essential oil.

Sl. No.	Phytoconstituents	Results
1	Tannins	+
2	Saponins	-
3	Flavonoid	+
4	Phenols	+
5	Protein	-
6	Carbohydrate	+
7	Glycosides	+

(+) indicates presence, (-) Indicates absence.

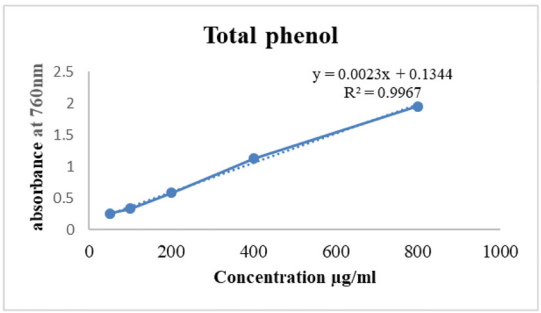

Fig. 2. Graphical representation of gallic acid.

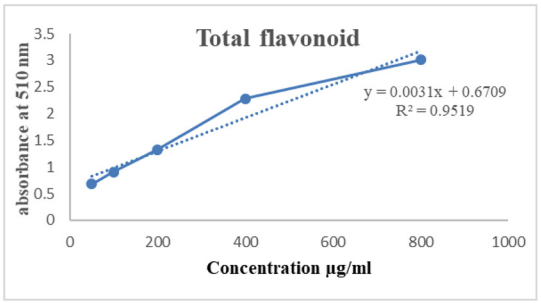

Fig. 3. Graphical representation of quercetin.

Determination of total phenols and flavonoids

The total phenolic and flavonoids contents in the sample are expressed in terms of gallic acid equivalent and quercetin equivalent respectively. The total phenol and flavonoid content were evaluated from the standard calibration graphs respectively (Figs. 2-3). Total phenolic content in the examined sample were 74.4 ± 0.06 mg/100g while total flavonoids content was 3.0±0.031mg/100g.

DPPH radical scavenging activity

DPPH free radical scavenging method was used to determine the concentration of essential oil at which they scavenge the 50% of the DPPH solution termed as IC_{50} value. The different concentrations showed different levels of radical scavenging activity of *Curcuma caesia* and the standard quercetin in the calibration graph (Fig. 4). So, therefore the sample

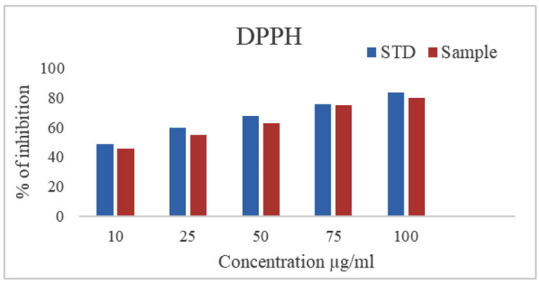

Fig. 4. Inhibition percentage of quercetin and *C. caesia* essential oil.

Table 2. Inhibition percentage and IC_{50} values of quercetin and *C. caesia* essential oil.

Concentration (µg/ml)	Inhibition percentage of STD quercetin	Inhibition percentage of essential oil	IC_{50} std	IC_{50} sample
10	48.6	45.7		
25	59.6	55		
50	67.7	62.7	5.9µg/ml	16µg/ml
75	75.7	74.9		
100	83.7	79.8		

can inhibit upto 79.8% at concentration of 100µl/ml and the standard quercetin can inhibit up to 83.7% at the same concentration (Table 2).

Antibacterial

The antibacterial activity of the sample varied according to concentration (Table 3) are shown in terms of millimeter. The greatest zone of inhibition of 18mm was recorded against *Vibrio cholerae* followed by 14mm and 10mm against *Staphylococcus aureus* and *Bacillus cereus*, respectively, and less than 10mm against *Salmonella typhimurium* and *E. coli* (Fig. 5).

Table 3. Antibacterial activity of *Curcuma caesia* rhizome essential oil.

Sl. No.	Bacteria	Zone inhibition (mm)		STD
		Oil	Oil + DMSO	(Amoxicillin)
1	*Staphylococcus aureus* (MTCC-6908)	14 mm	16 mm	21 mm
2	*Bacillus cereus* (MTCC-430)	10 mm	11 mm	32 mm
3	*Salmonella typhimurium* (MTCC-3224)	>10 mm	10 mm	18 mm
4	*E. coli* (MTCC-723)	>10 mm	10 mm	33 mm
5	*Vibrio cholerae* (MTCC-3906)	18 mm	14 mm	19 mm

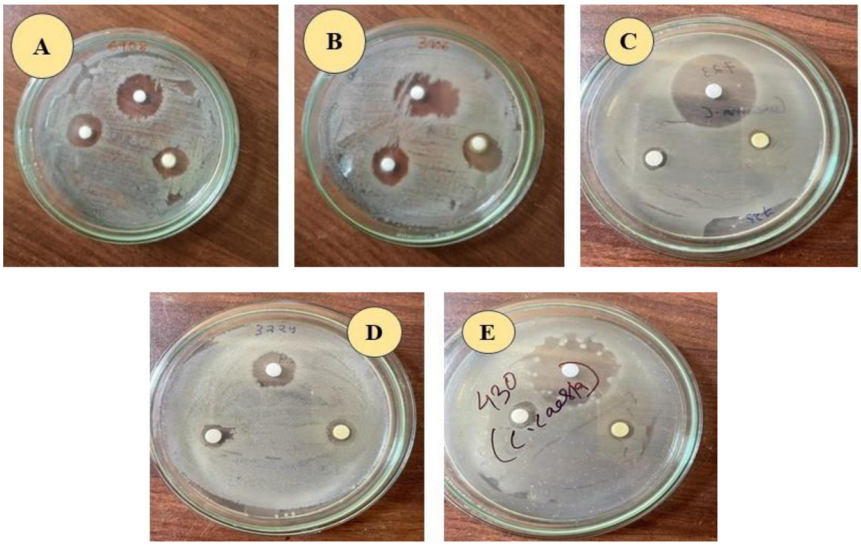

Fig. 5. (A) *Staphylococcus aureus*, (B) *Vibrio cholerae* ,(C) *E. coli,* (D) *Salmonella typhimurium,* (E) *Bacillus cereus.*

DISCUSSION

The use of medicinal plants is possibly the earliest technique of treating illness. They are quickly metabolized within the body and have no negative side effects, which leads to phytochemical-based therapies (Anetor *et al*. 2008). Plants generate a variety of secondary metabolites to defend themselves against exogenous biotic restrictions (Guerriero *et al.* 2018). These compounds have essential physiological and ecological impacts. Flavonoids and phenols are secondary metabolites found in plants. They contain an aromatic ring with at least one hydroxyl group. They are used in many pharmaceuticals due to their antioxidant, antimicrobial and anticancer effects (Sulaiman and Balachandran 2012). Tannin has been used as an active ingredient in medicine and beverages due to its antioxidant effects (Tong *et al.* 2022, Olejar *et al.* 2016). Tannins possess some of the biological properties such as anti-inflammatory, anticancer, antiallergic (Ghosh 2015).

Based on the present study it can be concluded that the *Curcuma caesia* rhizome oil was found to have different classes of compounds including phenols, flavonoids, tannins, glycosides and carbohydrates. Among these, flavonoids are the largest group of naturally occurring phenolic compounds found in different parts of the plant either in a free state and as glycosides. Moreover, essential oil is reported to have many biological activities including antimicrobial, mitochondrial adhesion inhibition, antiulcer, anti-arthritic, antiangiogenic, anticancer, protein kinase inhibition (Bhat *et al.* 2005).

Curcuma caesia has strong antioxidant properties, as shown by its ability to scavenge free radicals. This makes it an effective antioxidant that can protect biological systems against oxidative stress, which is a key factor in various diseases such as aging, cancer, diabetes, cardiovascular disorders, and rheumatoid arthritis. The presence of phenolic components in *Curcuma caesia* is believed to contribute to these beneficial effects. Overall, *Curcuma caesia* is a source of natural antioxidant that can be important in disease prevention and health preservation (Kalita *et al.* 2019). It appears that the presently examined *Curcuma caesia* rhizome oil was able to scavenge the free radical upto 79.8% as compared to the standard quercetin (83.7%) which might be attributed by the presence of natural antioxidant compounds, particularly plant secondary metabolites i.e., phenolic compounds and flavonoids which are generated by plant to defend themselves or to stimulate growth in difficult environments (Tungmunnithum *et al.* 2018). These free radical scavenging activities could be attributed to the presence of phenolics compounds which are key elements of *Curcuma rhizomes.* Phenolics and flavonoids are commonly known as the largest phytochemical molecules with antioxidant properties from plants (Zahoor *et al.* 2018, Tungmunnithum *et al.* 2018).

The antibacterial activity of *Curcuma caesia* essential oil might be attributed by the classes of compounds present in it. Flavonoids are well known as antibacterial agents against a wide range of pathogenic microorganism (Xie *et al.* 2015). The antimicrobial activity of the rhizomes of *Curcuma caesia* was evaluated by using the disk diffusion method. The microorganisms chosen to be studied are two gram-negative and two gram-positive bacteria viz., *Salmonella typhimurium* (MTCC 3224), *Escherichia coli* (MTCC 723), *Bacillus cereus* (MTCC 430) and *Staphylococcus aureus* (MTCC 6908) were used for the antibacterial activity. This microorganism were chosen to be studied as they are imperative pathogens and furthermore because of quickly created anti-microbial resistance. The plant extract possesses antimicrobial activity.

In conclusion, it can be said that our study indicates presence of various phytochemicals in *Curcuma caesia* and its ethnomedical claims for the traditional use of the plant in the treatment of inflammation was true according to the above experimental results. Therefore, it can be stated that the essential oil extract from *Curcuma caesia* Roxb. is not only rich in antioxidant activity but also has antibacterial activities. Further investigation in this direction may help in formulation of effective herbal antibacterial preparations from *Curcuma caesia* Roxb. To promote proper conservation sustainable use of such plant resources, awareness of local communities should be enhanced incorporating the traditional knowledge with scientific findings.

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