Bioactivity Screening of Thirty Black Turmeric (*Curcuma caesia* Roxb.) Essential Oils Against Free Radicals and MDR Isolates

Pharmacognosy Magazine
19(3) 615–625, 2023

DOI: 10.1177/09731296231174958
journals.sagepub.com/home/phm
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Abstract

Background: *Curcuma caesia* Roxb. (Black turmeric) is a perennial medicinal herb belonging to the *Zingiberaceae* family that is endangered in Southeast Asia. It is treasured for its high-quality essential oil with tremendous medicinal and aromatic properties. In the present scenario, *C. caesia* Roxb. is an unexplored plant for drug discovery.
Objectives: The present study was undertaken to compare the bioactivities of Thirty *C. caesia* rhizomes and leaf oils collected from various eco-regions of Eastern India.
Materials and Methods: The comparative antioxidant and antimicrobial activities of leaf and rhizome essential oils from different eco-regions of Eastern India were assessed. The antioxidant activities were evaluated against standards like butylated hydroxytoluene (BHT) and ascorbic acid by the 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assays. Moreover, the essential oils were also evaluated for their antimicrobial activity using the broth micro-dilution assay for minimum inhibitory concentrations (MIC) against multidrug resistant strains.
Results: Leaf essential oils exhibited a considerable level of antioxidant potential as compared to rhizome essential oils and standard BHT. Furthermore, the essential oils also possessed a significant level of inhibitory activity against three multidrug-resistant (MDR) strains, such as *Acinetobacter baumannii, Escherichia coli*, and *Klebsiella pneumoniae*. The rhizome essential oils had shown the most effective antimicrobial activity against *A. baumannii* (MIC: 0.09 to 6.25 µg/mL) when compared with the positive control, ampicillin (MIC: 25 µg/mL).
Conclusion: The variability in bioactivities was greatly influenced by geographical origin. The identified accessions of *C. caesia*, that is, Cc 26 with better bioactivity potential, might be useful for formulating drugs in the future.

Keywords

Curcuma caesia, essential oil, 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), multi-drug resistance, minimum inhibitory concentration

Received 16 December 2022; **accepted** 28 February 2023

Introduction

Herbs and spices have been recognized for their aromatic and health-boosting properties since time immemorial. The *Zingiberaceae* family includes plants that are known worldwide for their distinctive organoleptic properties (color, smell, and taste), which are often used as spices in the kitchen. In addition, these are also used in various industries like pharmaceutical, medical, and cosmetics due to their proven biological activities (Zhang et al., 2020). In the folklore medicinal system, the naturopaths used the genus *Curcuma* to deal with various diseases (Ivanović et al., 2021). Among six commonly used *Curcuma* species, including *C. longa* (turmeric), *C. caesia* (black turmeric), *C. zedoaria* (zedoary), *C. amada* (mango ginger), *C. angustifolia* (east Indian arrowroot), and *C. aromatica* (wild turmeric), *C. caesia* is

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known for its ethnomedical importance in the Northeast region of India (Tushar et al., 2010).

C. caesia Roxb. (black turmeric) belongs to the family *Zingiberaceae*, a rhizomatous aromatic herb with tremendous therapeutic and commercial value (Sahu et al., 2016). It is characterized by a bluish-black tuberous rhizome e with a strong camphoraceous odor and a deep violet-red patch on leaves that runs throughout the length of the lamina (Mahanta et al., 2023; Singh et al., 2021). Traditionally, rhizomes of *C. caesia* are used in the treatment of various ailments and metabolic disorders like leukoderma, asthma, tumors, piles, and bronchitis (Das et al., 2013). Moreover, the rhizome paste is also used for rheumatic pains and dysentery (Israr et al., 2012). However, the leaves are also used as plasters for lymphangitis, furunculosis, and adenitis (Donipati & Sreeramulu, 2015). It possesses various promising pharmacological activities like antimicrobial (Borah et al., 2019), antifungal (Banerjee & Nigam, 1976), antioxidant (Rajamma et al., 2012), anti-cancerous (Mukunthan et al., 2017), antiulcer (Tripathy & Afrin, 2016), genotoxicity (Paw et al., 2020), smooth muscle relaxant and antiasthmatic (Arulmozhi et al., 2006), anthelmintic (Gill et al., 2011), and thrombolytic (Fathima et al., 2015).

Essential oils (EOs) are a complex mixture of several constituents, including alcohols, aldehydes, esters, ketones, oxides, and terpenoids, used in the perfumery, aromatherapy, pharmaceuticals, and agrochemical industries (Sahu et al., 2016). EOs are concentrated natural products with ample secondary metabolites to counter pathogenic microbes as their survival strategy (Mahanta et al., 2022). EOs can also be used as a source of medicine in the pharmaceutical industry, as they contain many robust defense systems against pathogenic microbes (Sahu et al., 2016). Several phytochemical studies on EOs led to the identification of sesquiterpenoids and monoterpenoids as significant components that possess a wide variety of pharmacological properties (Mahanta et al., 2022). Therefore, the use of medicinal plants rich in EOs represents a viable source for the control of some diseases and could constitute a possible therapeutic alternative due to their effectiveness. In industries, these oils are widely studied, mainly for their potential applications as agents promoting biological activities (de Oliveira et al., 2018).

In general, oxidative stress-related diseases are more often exposed to mankind (Tanvir et al., 2017). In the field of pharmacology, antioxidants occupy a specific place as inhibitors of oxidative compounds by preventing oxidation (Kalia, 2005). Oxidative stress-related diseases are linked with the formation of strong free radicals, which play a specific role in the pathogenesis of various degenerative human ailments, including cancer, Alzheimer's, immune deficiency diseases, and asthma (Pham-Huy et al., 2008). Nowadays, people use commercially available synthetic antioxidants to mitigate oxidative stress. But in spite of this, intake of natural dietary antioxidants can also reduce free radical formation without any adverse effects (Sahoo et al., 2013). Given their reasonably safe reports, herbs are mainly used as an alternative source to increase demand and suggest the utilization of EOs as natural antioxidants (Karimi & Moradi, 2015). Moreover, many infectious diseases are difficult to treat due to their antibiotic resistance, which is becoming an alarming situation for humankind to combat infectious diseases. Hence, the necessity of developing effective antimicrobial agents is in rising demand.

Considering the ethnomedicinal importance of *C. caesia* essential oil, emphasis should be given to evaluate its variability in bioactivities across different geographical regions. As such, no research has been carried out on comparative bioactivities screening of the rhizome and leaf essential oils of *C. caesia* from Eastern India. Therefore, the current study was designed to screen the antioxidant and antimicrobial potential of *C. caesia* accessions collected from different eco-regions of Eastern India. This study will also assist in identifying *C. caesia* accessions with desirable bioactivities to add natural derivatives to cure stress-related diseases.

Materials and Methods

Plant Materials

Rhizomes and leaves of *C. caesia* were collected from different eco-regions of Eastern India (Table 1). Samples were identified by a taxonomist and deposited in the herbarium of the Centre for Biotechnology (CBT), Siksha O Anusandhan University, Bhubaneswar. Rhizomes were planted in the medicinal plant garden of CBT for further study.

Bacterial Strains

Three gram-negative, multidrug-resistant (MDR) bacterial strains (*Acinetobacter baumannii, Escherichia coli*, and *Klebsiella pneumoniae*) were isolated from the Department of Microbiology, Siksha 'O' Anusandhan (Deemed to be the University), Bhubaneswar.

Essential Oil Extraction

100 g of fresh leaf and rhizome samples of *C. caesia* were chopped and subjected to hydrodistillation using Clevenger apparatus for 3 and 6 h, respectively. The percentage of oil yield was calculated on the basis of fresh weight. The moisture trace present in essential oils (EOs) was removed by treating it with anhydrous sodium sulfate (Na_2SO_4) and stored in amber glass vials at 4°C until further analysis.

DPPH Radical Scavenging Activity

The DPPH radical scavenging activities of the obtained essential oils (EOs) were measured according to the protocol

Table 1. Geographical Characteristics of *C. caesia* Accessions Collected from Different Eco-regions of Eastern India.

Accession	Locality	Province	Longitude (E)	Latitude (N)	Altitude (m)
Cc1	Birbhum	West Bengal	87.3771°	23.50246°	45
Cc2	Sargachhi	West Bengal	88.14597°	24.01186°	23
Cc3	Krishnanagar	West Bengal	88.3050°	23.2432°	19
Cc4	Narendrapur	West Bengal	88.04227°	22.38495°	5
Cc5	South 24 Parganas	West Bengal	85.46309°	20.1718°	76
Cc6	Mayurbhanj	Odisha	86.2574°	22.00313°	322
Cc7	Nayagarh	Odisha	85.01240°	20.09556°	110
Cc8	Kandhamal	Odisha	84.0103°	20.0831°	565
Cc9	Gajapati	Odisha	84.1186°	19.11284°	719
Cc10	Koraput	Odisha	82.4449°	18.51218°	749
Cc11	Kalinga nagar, Bhubaneswar	Odisha	85.792°	20.2591°	58
Cc12	Khandagiri, Bhubaneswar	Odisha	85.781°	20.28°	58
Cc13	Old Town, Bhubaneswar	Odisha	85°50'02"	20°14'33"	22
Cc14	Patrapada, Bhubaneswar	Odisha	85°45'35"	20°14'36"	44
Cc15	Bokaghat	Assam	93.608°	26.642°	76
Cc16	Darrang	Assam	92.5000 °	26.7500°	102
Cc17	Dimapur	Nagaland	93°43'33"	25°54'39"	154
Cc18	Hojai	Assam	92.866402 °	26.006807°	59
Cc19	Jhargram	West Bengal	86.99243580 °	22.4370351°	81
Cc20	Kothavalasa, Araku Valley	Andhra Pradesh	82° 52'16"	18°19'24"	928
Cc21	Khammam	Andhra Pradesh	80.6327°	17.6351°	107
Cc22	Daringbadi	Odisha	84.74916°	19.54133°	915
Cc23	Tentulikhunti	Odisha	82.7232°	19.2951°	662
Cc24	Phulbani	Odisha	84.233147°	20.479712°	485
Cc25	Meghdoot Nagar	Madhya Pradesh	75.5420°	22.4524°	550
Cc26	Abhanpur	Chattisgarh	81.6603°	21.2516°	298
Cc27	Sainikwadi, Vadgansheri	Maharashtra	73.9169°	18.5510°	549
Cc28	Kandhamal	Odisha	84.0167°	20.1342°	700
Cc29	Ranchi	Jharkand	85.3096°	23.3441°	651
Cc30	Goalpara	Assam	90.6252°	26.1641°	35

Abbreviation: *C. caesia: Curcuma caesia.*

of Sahoo et al. (2014). A methanolic solution of EOs at different concentrations (1, 5, 10, 20, and 30 µg/mL) was added to 1 mL of 0.1 mM DPPH. The solutions were properly mixed and were kept at room temperature for 30 mins in the dark. DPPH scavenging activities were measured by taking absorbance at 517 nm in a UV-visible spectrophotometer. Ascorbic acid and butylated hydroxytoluene (BHT) were taken as positive controls. The solution containing methanol and DPPH was taken as control. The scavenging activities were expressed by calculating the percentage of inhibition (% of inhibition) and determined by the following equation:

$$\%\ of\ inhibition = \left[(Control\ absorbance - Sample\ absorbance) / Control\ absorbance\right] \times 100$$

A graph was plotted showing % of inhibition versus sample concentration (µg/mL) for determining the half-maximal inhibitory concentration (IC_{50}).

ABTS Radical Scavenging Activity

ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay was performed to determine the antioxidant ability of the rhizome and leaf EOs following the method reported by Sahoo et al. (2014). For the preparation of ABTS stock solution, 7 mM of ABTS solution was mixed with 2.45 mM of ammonium persulfate, followed by incubating the mixture for 16 h at 37°C in a dark condition. The ABTS solution was then diluted with methanol to obtain an OD of 0.70 ± 0.02 at 734 nm. Afterwards, 0.3 mL of various

concentrations (1, 5, 10, 20, and 30 µg/mL) of oils were prepared by mixing those with 3 mL of ABTS solution. The solution containing methanol and ABTS without the sample was considered blank. Ascorbic acid and BHT were used as positive controls. The percentage of inhibition of the ABTS radical was estimated using the same formula as that of the DPPH assay. The concentration of EO that could inhibit 50% of the ABTS free radical was determined as the IC_{50} value.

Antimicrobial Activities

Minimum Inhibitory Concentration (MIC)

According to Clinical and Laboratory Standards Institute (CLSI, 2013) guidelines and following the protocol of Dash et al. (2022), the minimum inhibitory concentration (MIC) of extracted EOs against three MDR strains was determined by the broth micro-dilution method. The antimicrobial potential was tested by Mueller Hinton Broth (MHB) against the three MDR strains. EOs (100 µL) with a concentration of 100 µg/mL were prepared by dissolving with dimethyl sulfoxide (DMSO). A bacterial culture with a concentration of 10^6 CFU/mL was adjusted from the overnight suspension. EOs were analyzed in a concentration range from 25 to 0.048 µg/mL by following a twofold serial dilution method with MHB in a 96-microtiter plate. The microtitre plate wells were suspended with 100 µL of prepared bacterial suspensions (10^6 CFU/mL). The inoculated plates were kept at 37°C for 24 h. Ampicillin was used as the control. For MIC estimation, 5 µL of 0.5M 2,3,5-triphenyl tetrazolium chloride (TTC) was added to each well and kept for 30 min of incubation at 37°C. The MIC was measured by the lowest concentration of the samples showing no coloration after adding TTC, which corresponds to the absence of visible microbial growth. Each MDR strain was tested in triplicate.

Minimum Bactericidal Concentration (MBC)

The same microdilution method was used to determine the minimum concentration required to kill the bacteria. After 24 h of incubation, a loop of culture from each well of the bacterial plates was inoculated on Mueller-Hinton Agar (MHA) medium and incubated at 37°C for 24 h. The MBC value is estimated as the minimum concentration of EOs resulting in 99.5% death of the inoculum.

Statistical Analysis

Statistical assays were carried out to obtain the mean oil yield and antioxidant potential of thirty different accessions. The mean oil yield and IC_{50} were calculated using one-way ANOVA followed by Tukey's HSD test at 95% of the confidence interval to reveal the significant difference in oil yield and antioxidant potential of different growing climates.

Results and Discussion

Essential Oil Yield

The fresh rhizome and leaf samples were hydro-distilled using Clevenger apparatus, and a noteworthy difference in oil yield percentage was observed. The mean oil yield of thirty different accessions was calculated using a one-way ANOVA followed by Tukey's HSD test at a 95% confidence interval. The mean oil yield of rhizome and leaf samples ranged between 0.15 ± 0.02 to 0.8 ± 0.02% and 0.1 ± 0.02 to 0.41 ± 0.05%, respectively, on a fresh weight basis (v/w) (Table 2). A remarkable difference in the oil yields was observed from the statistical analysis, possibly due to the variation in their growing areas of *C. caesia* accessions. The highest rhizome EO yield of 0.81 ± 0.02% (v/w) was found in the Cc26 accession, whereas the Cc30 accession showed the highest leaf EO yield (0.41 ± 0.05%). Various reports are available on the oil yield of rhizome samples (both fresh and dry) of *C. caesia* (Chaturvedi et al., 2021; Mukunthan et al., 2014). But our finding corroborates one of the earlier published studies by Angel et al. (2014), who reported an EO yield of 0.5% in fresh rhizomes from Kerala. However, till date, only one report is available on *C. caesia* leaf Eos in which a leaf oil yield of 0.7% (dry weight basis v/w) was observed in shade-dried leaves collected from Jorhat district, Assam (Donipati & Sreeramulu, 2015). Meanwhile, numerous research studies have also indicated a notable difference in rhizome EOs collected over different geographic locations (Angel et al., 2014; Chaturvedi et al., 2021; Mukunthan et al., 2014). The *C. caesia* rhizome EOs collected from Madhya Pradesh exhibited an oil yield of 1.5% (dry weight basis, v/w) reported by Chaturvedi et al. (2021). Likewise, the rhizome EO yield of 0.13% (dry weight basis, v/w) was obtained from Calicut (Mukunthan et al., 2014). This indicates that variability in oil yield depends on the use of fresh or dried samples and their geographical origin. Moreover, it is greatly influenced by various environmental entities (Xie et al., 2012).

Antioxidant Activity

Essential oils are complex mixtures of secondary metabolites with different functional groups, polarities, and chemical behaviors. So, relying on the results of the single assay can only give a reductive suggestion for antioxidant properties (Brewer, 2011). Therefore, a multiple-assay approach is highly advisable.

In the present study, the radical scavenging potential of *C. caesia* rhizome and leaf EOs was evaluated by two different *in vitro* assays, namely, DPPH and ABTS. The DPPH scavenging activity is based on the decolorization of methanolic DPPH by accepting the hydrogen radical from antioxidants to make DPPH more stable (DPPH-H) (Sahoo et al., 2012). The antioxidant potential is measured

Table 2. Essential Oil Yields of 30 *C. caesia* Accessions.

Accessions	Oil Yield (%)[a] (Mean ± SD)*	
	Rhizome Oil	Leaf Oil
Cc1	$0.6 \pm 0.015^{C,D}$	$0.13 \pm 0.02^{G,H,I}$
Cc2	$0.39 \pm 0.02^{J,K,L}$	$0.14 \pm 0.02^{F,G,H,I}$
Cc3	$0.55 \pm 0.015^{C,D,E,F,G}$	0.1 ± 0.02^{I}
Cc4	$0.4 \pm 0.02^{I,J,K,L}$	$0.17 \pm 0.02^{F,G,H,I}$
Cc5	$0.38 \pm 0.03^{J,K,L}$	$0.17 \pm 0.03^{F,G,H,I}$
Cc6	$0.64 \pm 0.03^{B,C}$	$0.15 \pm 0.02^{F,G,H,I}$
Cc7	$0.5 \pm 0.015^{E,F,G,H,I}$	$0.28 \pm 0.02^{B,C,D}$
Cc8	$0.58 \pm 0.02^{C,D,E,F}$	$0.13 \pm 0.02^{G,H,I}$
Cc9	$0.55 \pm 0.02^{C,D,E,F,G}$	$0.11 \pm 0.03^{H,I}$
Cc10	$0.45 \pm 0.02^{H,I,J}$	0.1 ± 0.02^{I}
Cc11	0.15 ± 0.02^{M}	$0.24 \pm 0.03^{D,E,F}$
Cc12	$0.65 \pm 0.03^{B,C}$	$0.35 \pm 0.03^{A,B,C}$
Cc13	$0.71 \pm 0.02^{A,B}$	$0.17 \pm 0.02^{F,G,H,I}$
Cc14	$0.58 \pm 0.04^{A,B}$	0.1 ± 0.015^{I}
Cc15	$0.36 \pm 0.02^{J,K,L}$	$0.12 \pm 0.02^{G,H,I}$
Cc16	$0.40 \pm 0.02^{I,J,K,L}$	$0.14 \pm 0.03^{F,G,H,I}$
Cc17	$0.41 \pm 0.03^{H,I,J,K}$	$0.16 \pm 0.03^{F,G,H,I}$
Cc18	$0.5 \pm 0.04^{E,F,G,H,I}$	$0.20 \pm 0.04^{D,E,F,G,H}$
Cc19	$0.41 \pm 0.04^{H,I,J,K}$	$0.14 \pm 0.03^{G,H,I}$
Cc20	$0.50 \pm 0.03^{D,E,F,G,H}$	$0.17 \pm 0.02^{F,G,H,I}$
Cc21	$0.45 \pm 0.02^{H,I,J}$	$0.27 \pm 0.02^{C,D,E}$
Cc22	$0.5 \pm 0.02^{F,G,H,I}$	$0.30 \pm 0.04^{B,C,D}$
Cc23	$0.34 \pm 0.03^{K,I}$	$0.17 \pm 0.02^{F,G,H,I}$
Cc24	$0.60 \pm 0.02^{C,D,E,F}$	$0.15 \pm 0.02^{F,G,H,I}$
Cc25	0.30 ± 0.02^{L}	$0.18 \pm 0.03^{E,F,G,H,I}$
Cc26	0.81 ± 0.02^{A}	$0.37 \pm 0.04^{A,B}$
Cc27	$0.46 \pm 0.015^{G,H,I,J}$	$0.17 \pm 0.02^{F,G,H,I}$
Cc28	$0.6 \pm 0.02^{C,D,E}$	$0.21 \pm 0.025^{D,E,F,G}$
Cc29	$0.72 \pm 0.02^{A,B}$	$0.27 \pm 0.03^{C,D,E}$
Cc30	0.75 ± 0.03^{A}	0.41 ± 0.05^{A}

Abbreviations: *C. caesia*: *Curcuma caesia*; SD: standard deviation.

Notes: [a]The essential oil yield is given in % (v/w) based on the fresh weight of the plant material.

*Mean having different letters in the column was significantly different as per Tukey's HSD test at $p < 0.05$.

in terms of the IC_{50} value. The lower the IC_{50} value, the higher the antioxidant potential of EOs. Rhizome and leaf EOs resulted in a considerable variation in their antioxidant potential over thirty accessions. The IC_{50} values for rhizome and leaf EOs ranged from 8.66 ± 0.02 to 22.53 ± 0.03 µg/mL and 8.31 ± 0.02 to 18.35 ± 0.015 µg/mL, respectively, and it has been observed that scavenging activity increases with an increase in concentration of EOs. The obtained results were represented by the mean ± standard deviation of triplicates using one-way ANOVA followed by Tukey's HSD test at 95% of the confidence interval (Table 3). Antioxidant potential was evaluated and compared with positive controls, that is, ascorbic acid and BHT, with respective IC_{50} values of 5.49 ± 0.02 µg/mL and 18.42 ± 0.02 µg/mL. Though, a study conducted by Paw et al., 2020 showed that the rhizome EO of *C. caesia* has IC_{50} value 48.08 µg/mL as compared to ascorbic acid (IC_{50}−149.1 µg/mL), which indicates that the rhizome EOs of Eastern India accessions have shown better scavenging activity with less IC_{50} values from 8.66 ± 0.02 to 22.53 ± 0.03 µg/mL.

Table 3. IC_{50} Values of Rhizome and Leaf Essential Oils of *C. caesia* by DPPH and ABTS Scavenging Assay.

	DPPH Assay IC_{50} (µg/mL) (Mean ± SD)*		ABTS Assay IC_{50} (µg/mL) (Mean ± SD)*	
Accessions and Controls	Rhizome Oil	Leaf Oil	Rhizome Oil	Leaf Oil
Cc1	11.95 ± 0.02^{O}	9.8 ± 0.015^{M}	10.52 ± 0.02^{R}	10.55 ± 0.02^{K}
Cc2	15.33 ± 0.04^{J}	12.34 ± 0.03^{G}	13.87 ± 0.01^{L}	12.93 ± 0.03^{H}
Cc3	12.12 ± 0.02^{N}	10.84 ± 0.02^{J}	14.65 ± 0.03^{I}	13.78 ± 0.015^{E}
Cc4	12.56 ± 0.02^{M}	10.61 ± 0.03^{K}	10.73 ± 0.02^{Q}	10.34 ± 0.03^{L}
Cc5	15.44 ± 0.04^{I,J}	12.12 ± 0.03^{H}	18.42 ± 0.02^{E}	16.65 ± 0.03^{D}
Cc6	10.85 ± 0.02^{P}	8.6 ± 0.03^{Q}	9.96 ± 0.02^{S}	8.24 ± 0.02^{Q,R}
Cc7	16.17 ± 0.02^{G}	12.67 ± 0.03^{F}	14.26 ± 0.02^{K}	12.54 ± 0.02^{I}
Cc8	18.54 ± 0.03^{D}	15.15 ± 0.03^{C}	20.33 ± 0.02^{D}	19.7 ± 0.02^{A}
Cc9	14.54 ± 0.03^{K}	10.17 ± 0.02^{L}	15.64 ± 0.02^{F}	13.65 ± 0.03^{F}
Cc10	16.33 ± 0.02^{F}	11.75 ± 0.02^{I}	14.85 ± 0.04^{H}	13.43 ± 0.03^{G}
Cc11	8.7 ± 0.03^{T}	8.54 ± 0.03^{Q,R}	7.74 ± 0.02^{Y}	7.36 ± 0.015^{T}
Cc12	9.3 ± 0.03^{S}	8.95 ± 0.02^{O,P}	8.46 ± 0.04^{X}	8.38 ± 0.03^{P}
Cc13	10.16 ± 0.04^{Q}	9.6 ± 0.02^{N}	9.22 ± 0.02^{V}	8.17 ± 0.02^{R}
Cc14	22.01 ± 0.03^{C}	17.22 ± 0.015^{B}	21.94 ± 0.03^{B}	16.56 ± 0.02^{D}
Cc15	14.41 ± 0.03^{K}	10.07 ± 0.03^{L}	14.24 ± 0.03^{K}	9.35 ± 0.02^{N}
Cc16	27.42 ± 0.02^{A}	18.35 ± 0.04^{A}	26.85 ± 0.04^{A}	17.56 ± 0.015^{C}
Cc17	16.74 ± 0.03^{E}	11.75 ± 0.02^{I}	15.24 ± 0.03^{G}	10.85 ± 0.02^{J}
Cc18	13.06 ± 0.02^{L}	9.01 ± 0.02^{O}	12.54 ± 0.03^{M}	8.96 ± 0.02^{O}
Cc19	11.86 ± 0.02^{O}	8.54 ± 0.02^{Q,R}	10.86 ± 0.04^{P}	8.44 ± 0.02^{P}
Cc20	10.81 ± 0.015^{P}	9.85 ± 0.02^{M}	10.54 ± 0.03^{R}	9.44 ± 0.015^{N}
Cc21	12.44 ± 0.03^{M}	10.71 ± 0.03^{K}	11.85 ± 0.04^{N}	10.22 ± 0.02^{M}
Cc22	15.95 ± 0.015^{H}	13.33 ± 0.02^{E}	14.56 ± 0.04^{I,J}	12.54 ± 0.02^{I}
Cc23	12.55 ± 0.03^{M}	10.85 ± 0.04^{J}	11.95 ± 0.015^{N}	10.23 ± 0.02^{L,M}
Cc24	9.37 ± 0.03^{S}	8.45 ± 0.02^{R}	9.34 ± 0.015^{U}	8.23 ± 0.02^{Q,R}
Cc25	22.53 ± 0.03^{B}	14.55 ± 0.02^{D}	21.54 ± 0.02^{C}	13.54 ± 0.03^{F,G}
Cc26	8.66 ± 0.02^{T}	8.31 ± 0.02^{S}	8.64 ± 0.02^{W}	7.92 ± 0.02^{S}
Cc27	15.44 ± 0.03^{I}	11.85 ± 0.03^{I}	14.52 ± 0.02^{J}	10.24 ± 0.03^{L,M}
Cc28	12.55 ± 0.02^{M}	10.85 ± 0.02^{J}	11.53 ± 0.02^{O}	10.32 ± 0.02^{L,M}
Cc29	9.57 ± 0.02^{R}	8.52 ± 0.02^{Q,R}	9.52 ± 0.02^{T}	8.34 ± 0.02^{P,Q}
Cc30	10.85 ± 0.02^{P}	8.87 ± 0.015^{P}	10.77 ± 0.02^{P,Q}	8.45 ± 0.02^{P}
BHT**	18.42 ± 0.02^{D}	18.42 ± 0.02^{A}	18.42 ± 0.02^{E}	18.42 ± 0.02^{B}
Ascorbic acid**	5.4 ± 0.02^{U}	5.4 ± 0.02^{T}	5.4 ± 0.02^{Z}	5.4 ± 0.02^{U}

Abbreviations: *C. caesia*: *Curcuma caesia*; BHT: butylated hydroxytoluene.

Notes: *IC_{50} value with different letters in the column was significantly different as per Tukey's HSD test at $p < 0.05$.

**BHT and Ascorbic acid were used as positive controls.

The leaf EOs have also shown better antioxidant potential, with IC_{50} values ranging from 8.31 ± 0.02 to 18.35 ± 0.015 µg/mL compared to rhizome EOs and reference standard BHT (Figure 1). However, one report has demonstrated the free radical scavenging activity of *C. caesia* leaf EOs using the DPPH assay, and they were found to have excellent antioxidant potential, showing an IC_{50} value of 1.487 µg/mL (Borah et al., 2019). It has also been observed that among all 30 accessions, the Cc26 accession with rhizome and leaf EOs possessed the highest DPPH radical scavenging potential with IC_{50} values of 8.66 ± 0.02 µg/mL and 8.31 ± 0.02 µg/mL, respectively.

The antioxidant ability of rhizome and leaf EOs was also evaluated using the ABTS scavenging assay, which is based on the decolorization principle of the radical monocation of ABTS (ABTS*+) (Sahoo et al., 2012). This method for

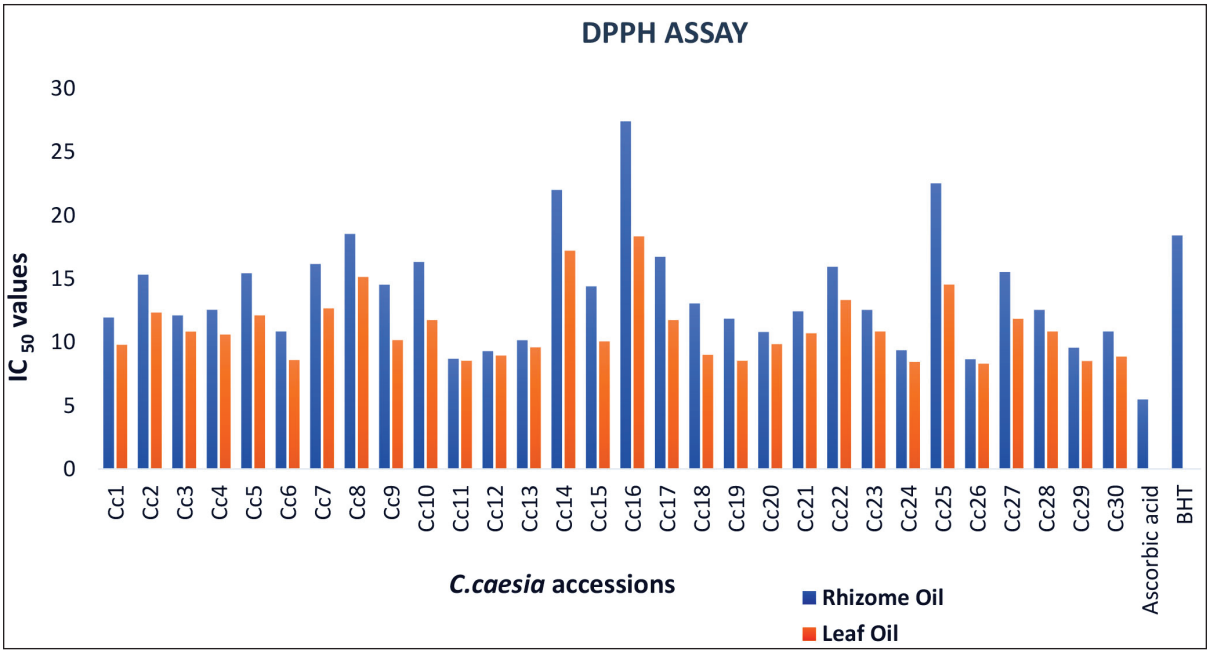

Figure 1. DPPH Radical Scavenging Activity of 30 *C. caesia* EOs and Standards (Ascorbic Acid and BHT)

Abbreviations: *C. caesia*: *Curcuma caesia*; EOs: essential oils; BHT: butylated hydroxytoluene.

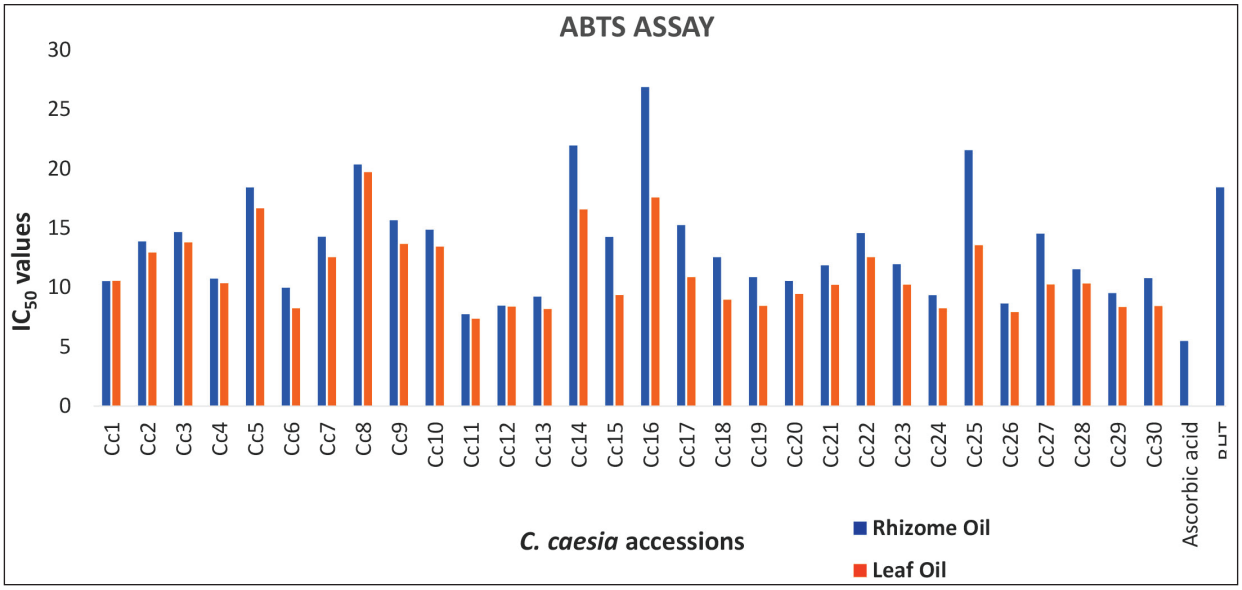

Figure 2. ABTS Radical Scavenging Activity of 30 *C. caesia* EOs and Standards (Ascorbic Acid and BHT)

Abbreviations: *C. caesia*: *Curcuma caesia*; BHT: butylated hydroxytoluene; EOs: essential oils.

screening antioxidant activity applies to both lipophilic and hydrophilic antioxidants, including flavonoids, carotenoids, plasma antioxidants, and hydroxycinnamate (Re et al., 1999). All the accessions showed similar results to those of the DPPH assay. Leaf EOs (7.92 ± 0.02 to 17.56 ± 0.015 µg/mL) have also shown prominent ABTS scavenging activity with low IC_{50} values as compared to rhizome EOs (8.46 ± 0.015 to 18.4 ± 0.02 µg/mL) and BHT (18.42 ± 0.02 µg/mL) (Figure 2). Even though ABTS and DPPH are based on a similar principle, the IC_{50} value of ABTS was less than that of DPPH, as illustrated in Table 3. Overall, accession Cc26 showed the best scavenging activity in both assays and may provide a

Table 4. MIC (µg/mL) and MBC (µg/mL) of *C. caesia* Rhizome and Leaf Samples against MDR Strains.

	Micro-organisms											
	K. pneumoniae				*E. coli*				*A. baumannii*			
Accessions	Rhizome Oil		Leaf Oil		Rhizome Oil		Leaf Oil		Rhizome Oil		Leaf Oil	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Cc1	12.5	25	6.25	12.5	6.25	12.5	6.25	6.25	0.78	1.56	1.56	3.12
Cc2	12.5	12.5	6.25	6.25	6.25	6.25	6.25	12.5	0.39	0.78	3.12	6.25
Cc3	6.25	6.25	3.12	6.25	6.25	12.5	6.25	6.25	0.39	0.78	0.78	1.56
Cc4	12.5	25	6.25	6.25	6.25	6.25	6.25	12.5	0.19	0.19	1.56	3.12
Cc5	6.25	12.5	3.12	3.12	6.25	6.25	6.25	6.25	0.19	0.39	1.56	1.56
Cc6	6.25	6.25	3.12	3.12	12.5	25	12.5	25	0.39	0.78	3.12	6.25
Cc7	12.5	25	6.25	12.5	12.5	12.5	12.5	25	0.39	0.39	1.56	1.56
Cc8	12.5	12.5	6.25	12.5	6.25	12.5	6.25	6.25	0.39	0.78	1.56	3.12
Cc9	6.25	6.25	3.12	3.12	6.25	6.25	6.25	6.25	0.39	0.39	0.78	1.56
Cc10	6.25	12.5	3.12	3.12	12.5	25	12.5	25	6.25	12.5	12.5	25
Cc11	6.25	12.5	3.12	6.25	6.25	6.25	6.25	6.25	0.39	0.39	6.25	12.5
Cc12	6.25	12.5	3.12	6.25	6.25	12.5	6.25	12.5	0.19	0.39	3.12	6.25
Cc13	12.5	12.5	6.25	6.25	6.25	6.25	6.25	6.25	0.78	1.56	3.12	6.25
Cc14	6.25	6.25	3.12	6.25	6.25	12.5	6.25	12.5	6.25	12.5	12.5	25
Cc15	12.5	12.5	6.25	12.5	6.25	12.5	6.25	6.25	0.39	0.78	6.25	6.25
Cc16	12.5	25	6.25	12.5	12.5	25	12.5	25	6.25	12.5	6.25	6.25
Cc17	12.5	12.5	6.25	6.25	12.5	25	12.5	12.5	0.39	0.78	3.12	3.12
Cc18	12.5	12.5	6.25	12.5	6.25	6.25	6.25	12.5	0.19	0.39	0.78	1.56
Cc19	6.25	12.5	3.12	6.25	6.25	6.25	6.25	6.25	0.78	1.56	3.12	3.12
Cc20	6.25	6.25	3.12	6.25	6.25	12.5	6.25	12.5	6.25	12.5	12.5	25
Cc21	12.5	12.5	6.25	6.25	6.25	6.25	6.25	6.25	0.19	0.39	6.25	6.25
Cc22	6.25	6.25	6.25	12.5	12.5	12.5	12.5	25	0.39	0.78	6.25	12.5
Cc23	12.5	25	6.25	6.25	6.25	12.5	6.25	6.25	0.39	0.39	6.25	12.5
Cc24	6.25	6.25	3.12	3.12	12.5	12.5	12.5	25	0.39	0.39	3.12	6.25
Cc25	6.25	12.5	3.12	6.25	6.25	12.5	6.25	6.25	0.39	0.39	0.78	1.56
Cc26	3.12	6.25	1.56	1.56	3.12	6.25	6.25	6.25	0.09	0.18	0.78	0.78
Cc27	12.5	12.5	6.25	12.5	6.25	12.5	6.25	12.5	0.39	0.39	6.25	12.5
Cc28	12.5	25	6.25	6.25	6.25	6.25	6.25	6.25	0.19	0.19	6.25	6.25
Cc29	12.5	25	6.25	12.5	12.5	12.5	12.5	25	0.78	0.78	6.25	6.25
Cc30	6.25	12.5	3.12	3.12	6.25	12.5	6.25	6.25	6.25	6.25	12.5	25
Ampicillin (Control)	25				3.12				0			

Abbreviations: *A. baumannii*: *Acinetobacter baumannii; E. coli*: *Escherichia coli; K. pneumoniae*: *Klebsiella pneumoniae; C. caesia*: *Curcuma caesia*; MDR: multidrug-resistant; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration.

source of natural antioxidants, which need an in-depth study to replace synthetic antioxidants.

Though limited information was available on the free radical scavenging activity, this study was compared with the rhizome extract of *C. caesia*. Liu et al. (2013) reported that methanolic extract of *C. caesia* rhizome showed good antioxidant ability, as compared to *C. aeruginosa* and *C. zedoaria*. Krishnaraj et al. (2010) also studied the antioxidant potential of rhizome extract, which is better than that of *C. amada.* Another study on the antioxidant activity of oleoresin isolated from *C. caesia* noted an IC_{50} value of 0.32 mg (Rajamma et al., 2012).

The present study is the first-ever report revealing variation in antioxidant potential among different *C. caesia* accessions from diverse eco-regions of Eastern India, and the significant variations in antioxidants may be observed due to environmental and seasonal variations (Hussain et al., 2008).

Antimicrobial Activity

The antimicrobial activity of *C. caesia* rhizome and leaf EOs was tested against three MDR strains (*A. baumannii, E. coli*, and *K. pneumoniae*) by measuring MIC and minimum bactericidal concentration (MBC) to compare with the

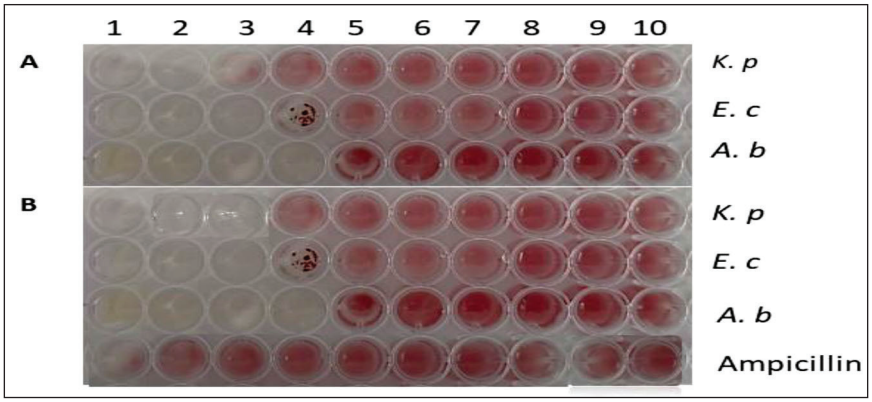

Figure 3. MIC Results of Rhizome (A) and Leaf (B) EO of *C. caesia* against MDR Isolates Well No. 1-10 Have the Concentration of 25 µg/mL to 0.097 µg/mL, Respectively.

Abbreviations: *C. caesia*: *Curcuma caesia; A. baumannii*: *Acinetobacter baumannii; E. coli*: *Escherichia coli; K. pneumoniae*: *Klebsiella pnumoniae;* MDR: multidrug-resistant; EO: essential oil; MIC: minimum inhibitory concentrations.

commercially available antibiotic standard Ampicillin (Table 4). Based on the results of MIC and MBC, EOs from various accessions displayed inhibitory growth with variable degrees of antimicrobial activity (Figure 3). The most significant activity was manifested by the rhizome EOs of *C. caesia* accessions against *A. baumannii*, with MIC and MBC values ranging from 0.09−6.25 µg/mL to 0.18−12.5 µg/mL, respectively. However, the leaf EOs performed better than the rhizome EOs for *K. pneumoniae*, with MIC and MBC values ranging from 3.12−6.25 µg/mL to 1.56−12.5 µg/mL. Comparatively, the standard Ampicillin has shown 25 µg/mL of both MIC and MBC values for *K. pneumoniae*, which signifies that leaf EOs is more susceptible than the antibiotic Ampicillin against this particular MDR strain. However, no report has been available on antimicrobial potential of *C. caesia* EOs against three MDR strains taken in this study, that is, *A. baumannii, E. coli*, and *K. pneumoniae*. So, this study has compared with rhizome and leaf EOs of other bacteria and fungus strains. Borah et al. (2019) reported leaf EOs of *C. caesia* to have the best bacteriostatic activity against *Staphylococcus aureus* with a MIC value of 2.5 µL and the fungi *Aspergillus niger*, with a MIC value of 5 µL. Formerly, a study conducted by Banerjee and Nigam (1976) reported that *C. caesia* rhizome EOs proved dynamic antifungal activity against *Aspergillus niger Caulophillus oryzae,* and *Aspergillus flavus*. Rajamma et al. (2012) found that oleoresins isolated from *C. caesia* rhizome EOs inhibited the growth of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and also shown best inhibitory action against *B. subtilis*.

Considering the above results, it can be assumed that *C. caesia* leaf EOs are more needed for finding antimicrobial agents against *K. pneumoniae* compared with ampicillin. The MBC values of EOs are almost similar or somewhat close to their respective MICs, elucidating that EOs can be used in order to identify promising bioresources in pharmaceutical products. For the first time, this work has been designed to study the variation with respect to antimicrobial activity among different *C. caesia* accessions collected from Eastern India. This variation may lead to the discovery of new potent antimicrobial agents for the pharma sector.

Conclusion

As people have become more techno-savvy, their blind dependency on commercial products will never end. But people should become more conscious toward nature to know the therapeutic importance of medicinal plants. Based on anecdotal evidence, *C. caesia* is the treasurer of incredible medicinal and aromatic properties. So, more focus should be given to its conservation and mass cultivation to fulfill the demands of pharmaceutical companies. The market value and mass cultivation can be increased by exploring its ethnopharmacological importance. Therefore, the current study was performed on the comparative bioactivity screening of *C. caesia* EOs. The EOs exhibited good antioxidant potential, even more than the commercial standard, that is, BHT and significant antimicrobial activity was also evaluated against three gram-negative MDR strains. It was observed that the rhizome EOs of *C. caesia* have shown the highest resistance against *A. baumannii*. Accordingly, this study summarized the importance of EOs, which can be used as a potent natural antioxidant, antimicrobial source, and formulating drug in the near future. It may also provide a new source for the pharma-industrial sectors. So, the ethnobotanical importance of this plant needs to be explored more for global development.

Summary

C. caesia Roxb. (Black turmeric) is known for its high-quality essential oil with tremendous medicinal and aromatic properties. Traditional healers used the genus *Curcuma* to treat various diseases, but in the present scenario, *C. caesia* Roxb. is very little known and almost untouched for drug discovery. Therefore, the present investigation has been designed to study the comparative bioactivity of thirty *C. caesia* rhizome and leaf essential oils (EOs) collected from Eastern India. The extracted essential oils of *C. caesia* have shown a considerable variation in oil yields, ranging from 0.15 ± 0.02% to 0.81 ± 0.02% and 0.10 ± 0.02% to 0.41 ± 0.05% (v/w, fresh weight basis) for both rhizomes and leaves, respectively. The antioxidant potential was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) as well as 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and compared with two standards like BHT and ascorbic acid. Leaf essential oils exhibited a considerable level of antioxidant potential as compared to rhizome essential oils and BHT. Thus, essential oils can be an alternative source of natural antioxidants. Furthermore, the essential oils also possessed a significant inhibitory activity against three MDR strains, such as *A. baumannii, E. coli*, and *K. pneumoniae*. The rhizome essential oils showed the most effective antimicrobial activity against *A. baumannii* (MIC: 0.09 to 6.25 µg/mL) when compared with Ampicillin (MIC: 25 µg/mL). Many reports are available regarding the bioactivity study of *C. caesia*, but no reports are found so far about the comparative evaluation of leaf and rhizome oils from *C. caesia* accessions collected from different regions of Eastern India. So, the current study has been designed to screen 30 *C. caesia* accessions for their antioxidant and antimicrobial potential to identify the suitable accessions of Eastern India with desirable bioactivities. As a result, the identified Cc-26 accession (from Abhanpur) with high essential oil yield and better bioactivity potential might be useful for both the grower and pharmaceutical industries.

Abbreviations

C. caesia; Curcuma caesia; EOs: essential oils; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); BHT: butylated hydroxytoluene; MDR: multidrug-resistance; *A. baumannii; Acinetobacter baumannii; E. coli; Escherichia coli; K. pneumoniae; Klebsiella pneumoniae*; MIC: minimum inhibitory concentration; DMSO: dimethyl sulfoxide; MHB: Mueller–Hinton Broth; TTC: 2,3,5-triphenyl tetrazolium chloride; MHA: Mueller–Hinton Agar; MBC: minimum bactericidal concentration.

Acknowledgments

The authors are thankful to SERB, DST, Government of India for providing financial support (Ref. No: SRG/2020/002425) for this work. The authors are also thankful to the President of Siksha O Anusandhan Deemed to be University for providing the necessary facilities to carry out this work.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship and/or publication of this article: SERB, DST, Government of India (Ref. No: SRG/2020/002425)

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Statement of Informed Consent and Ethical Approval

Not applicable.

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