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Profiling the Aroma Diversity in *Curcuma caesia* Roxb. Indigenous to Manipur, India

Takhelmayum Priyadini Devi[1], Bedabati Chowdhury[2*], Md Aminul Islam[3], Sudarshana Borah[4]

[1,2]Department of Botany, University of Science and Technology Meghalaya, Ri-Bhoi, Meghalaya-793101, India
[3]Department of Botany, Majuli College, Majuli, Assam-785106, India
[4]School of Pharmaceutical Sciences, University of Science and Technology Meghalaya, Ri-Bhoi, Meghalaya-793101, India

[*] **Corresponding author**
Dr. Bedabati Chowdhury
Associate professor, Department of Botany, University of Science and Technology Meghalaya, Ri-Bhoi, Meghalaya-793101, India
bedabatidasgupta6@gmail.com

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ABSTRACT:

Curcuma caesia (Kali Haldi), a rare and essential therapeutic herb in the Zingiberaceae family, has long been recognised in indigenous medicine for its healing properties. This study investigates the chemical profile of the volatile rhizome oil obtained from four accessions of *Curcuma caesia* collected from Manipur using Gas Chromatography-Mass Spectrometry (GC-MS). A total of fifty chemical constituents were detected, representing 87.04%-96.99% of the overall essential oil. The predominant constituents included epicurzerenone (11.22-41.44%),5,8-Dihydroxy-4a-methyl-4,4a,4b,5,6, 7,8,8a,9,10-decahydro-2(3H)-phenanthrenone (2.68-16.14%), cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1α,2β,4β)]- (4.48-10.85%), curcu manol (6.60-9.84%), benzofuran,6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-,trans- (6.58-9.84%), (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydrona phtho[2,3-b]furan-4(6H)-one (7.69-9.74%), β-Cyclocostunolide (4.99-9.46%), eucalyptol (3.38-8.78%), camphor (2.43-7.97%), etc. The present research reports some major components, such as (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one, curdione, and reynosin for the first time in *C. caesia* essential oil, highlighting significant geographical variations. The unique chemical profile of *C. caesia* from Manipur opens up new avenues for drug development and herbal remedies.

Keywords: Chemical profile. Curdione. Epicurzerenon e. GC/MS analysis. Hydrodistillation.

1. Introduction

Curcuma caesia Roxb., popularly referred to as 'Black Turmeric', is a perennial herb indigenous to North-East and Central India, with distribution in Thailand, Malaysia, Nepal, China, and Bangladesh (Lal et al., 2022). Locally referred to as 'Yaimu' in Manipuri, this species is a member of the Zingiberaceae family and reaches a height of 0.5-1.0 m. It is characterised by its huge tuberous rhizomes, wide oblong leaves, and pale yellow flowers with reddish borders (Ibrahim et al., 2023). The species is diploid with 42 chromosomes and is considered endangered due to biopiracy (Mahato and Sharma, 2018; Mukunthan et al.,

2014) . *C. caesia* has garnered considerable attention owing to its diverse array of bioactive compounds and potential therapeutic properties. Its rhizomes are known for their strong camphorous odour and bitter taste, indicating the presence of camphor, phenolics, curcuminoids, flavonoids, proteins, amino acids, alkaloids, and volatile oils (Borah et al., 2019). Conventionally, the rhizomes are used to treat various ailments, including wounds, contusions, enlargement of the spleen, snake bites, epileptic seizures, joint pain, toothache, ulcer, asthma, tumours, allergic eruptions, and gastric stress (Mahato and Sharma, 2018; Pandey et al., 2022). Therapeutically, the rhizome of this plant possesses the properties of anticancer, antioxidant, antiulcer, anti-inflammation, antifungal, antibacterial, anthelmintic, and smooth muscle relaxant (Baghel et al., 2013; Pandey et al., 2022).

Earlier analyses of *C. caesia* rhizome volatile oil from different regions have identified various compounds. For instance, the rhizomes from Madhya Pradesh showed camphor (28.3 %), ar-turmerone (12.3 %), (*Z*)-*β*-Ocimeme (8.2 %), ar-curcumene (6.8 %), 1,8-cineole (5.3 %), *β*-Elemene (4.8 %), borneol (4.4 %), and bornyl acetate (3.3 %) as major compounds (Pandey and Chowdhury, 2003). Similarly, in Calicut, thirty-five compounds were identified, with tropolone (15.86 %) as the predominant compound (Mukunthan et al., 2014). Furthermore, forty-eight compounds were identified from the rhizomes grown in Uttar Pradesh, with cycloisolongifolene,8,9-dehydro-9-formyl (11.67%), camphor (6.05%), eucalyptol (1,8-cineole) (5.96), and β-Germacrene (5.23%) as major components (Kumar et al., 2020). Borah et al.(2020) reported androsta-1,4-dien-3-one,17- (acetyloxy)-, (17.beta.) santanol acetate (16.11%), eucalyptol (12.98%), cycloprop[e]indene-1a,2(1H) dicarboxaldehyde,3a,4,5,6,6a,6b-hexahydro- 5,5,6b-trimethyl, (1a. alpha., 3a. beta., 6a. beta., 6b. alpha) (8.96%), methyl 7,12-octadecadienoate (6.75%), and (+)-2-Bornanone (6.60%) as principal constituents from the essential oil of *C. caesia* rhizome collected from Arunachal Pradesh. However, despite these thorough investigations, the volatile content of *C. caesia* from Manipur, North-East India, remains unexplored. Thus, this study aims to analyse the chemical profile of *C. caesia* rhizomes from this region.

2. Experimental

Plant Material

Four genotypes of fresh *Curcuma caesia* rhizomes were procured from distinct locations in Manipur. The location details are shown in Table 1. Figure 1 depicts representative pictures of intraspecific variation in four accessions.

Figure 1: Rhizome colour variation in four accessions of *C. caesia*

Table 1: Location details of *Curcuma caesia* Roxb. accessions collected from Manipur

Sl. No.	Accession	Place of Collection	Latitude (N)	Longitude (E)	Altitude (m)
1	CMP005	Chothe village, Bishnupur	24°36’28”	93°44’44”	833
2	CMP006	Kakching	24°32’32”	93°57’55”	794
3	CMP009	Litan, Chandel	24°26’49”	93°57’34”	791
4	CMP025	Senapati	25°15’38”	94°0’49”	1103

Extraction of Essential Oil

The volatile oil was isolated from fresh rhizomes of *C. caesia* using the hydro-distillation method employing a Clevenger apparatus for 3 hours. Approximately 250 g of fresh rhizomes were utilised for this process. The extracted volatile oil was then collected, desiccated with anhydrous sodium sulphate, and kept in an amber-coloured vial at 4°C until GC-MS analysis was conducted. The percentage of the essential oil yield was calculated using the formula given in Equation 1:

$$\text{Percentage of essential oil \%} = \frac{\text{Weight of oil(ml)}}{\text{Weight of sample (g)}} \times 100 \qquad (1)$$

Gas chromatography–Mass spectrometry analysis

The extracted rhizome volatile oil was analysed using a TRACE 1300 Gas Chromatograph coupled with an ISQ 7000 Mass spectrometer (Thermo Fisher Scientific, USA). The GC was fitted with a capillary column (30 m x 0.25 mm diameter). Helium served as a carrier gas at a flow rate of 1 mL/min. A 1 µL of diluted essential oil was introduced into the injector, which was kept at a temperature of 290°C. The mass scan range was 50-650 amu, and the ionisation energy of 70.5 eV was maintained. The different essential oil components were identified by matching their recorded mass spectra peaks with those in the Mainlib spectral library provided by the instrument software.

3. Result and Discussion

The hydro-distillation of *C. caesia* rhizomes obtained a viscous volatile oil with a yellowish to light purplish hue. Accession CMP005 yielded the highest oil at 0.6%, followed by CMP025 at 0.4%, CMP009 at 0.32%, and CMP006 at 0.18%. The results closely align with previous reports on the volatile oil yields from *C. caesia* (Angel et al., 2014 ;Singhet al.,2021; Vidya et al., 2023). The maximum yield of *C. caesia* has been reported as up to 1.5% (Pandey and Chowdhury, 2003).

The chemical composition, molecular structure of some major compounds, and chromatograms of the essential oil are presented in Table 2, Figure 2, and Figure 3. A total of fifty chemical constituents were identified, comprising 87.04%-96.99% of the total oil content. Among the studied genotypes, CMP005 exhibited the highest total area percentage of identified compounds (96.99%), while CMP006 had the lowest (87.04%). Among the identified constituents, epicurzerenone was the predominant compound, with concentrations ranging from 11.22% in genotype CMP005 to 41.44% in CMP025. This was followed by 5,8-Dihydroxy-4a-methyl-4,4a,4b,5,6,7,8,8a,9,10-decahydro-2(3H)-phenanthrenone, which ranged from 2.68% in CMP005 to 16.14% in CMP009. Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1α,2β,4β)]- content varied from 4.48% in CMP009 to 10.85% in CMP006. Curcumenol ranged from 6.60% in CMP009 to 9.84% in CMP025, benzofuran,6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-,trans- from 6.58% in CMP005 to 9.84% in CMP025, (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one from 7.69% in CMP009 to 9.74% in CMP005, β-Cyclocostunolide from 4.99% in CMP005 to 9.46% in CMP009, eucalyptol from 3.38% in CMP006 to 8.78% in CMP009, camphor from 2.43% in CMP025 to 7.97% in CMP009, and γ –Elemene from 2.93% in CMP009 to 6.65% in CMP006.

The component 4,4′-Dimethyl-2,2′-dimethylenebicyclohexyl-3,3′-diene was found only in genotype CMP005. Curdione (12.7%), tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene,trans-2,9-anti-9,10-trans-1,10- (10.39%), humulene (5%), caryophyllene (4.4%), and cyclohexane,1,2,3-trimethyl- (4.07%) were observed only in genotype CMP006. 3,5,8a-Trimethyl-4,4a,8a,9-tetrahydronaphtho[2,3-b]furan (5.07%) and (4aR,5S)-1-Hydroxy-4a,5-dimethyl-3-(propan-2-ylidene)-4,4a,5, 6-tetrahydronaphthalen-2(3H)-one (4.47%) were recorded only in CMP009. Reynosin (8.7%) was observed in CMP025.

The volatile oil composition of *C. caesia* rhizome differed greatly from earlier reported studies, possibly attributable to geographical variations. Notably, the analysed rhizome oil lacks cis-β-ocimene as compared to studies reported by Sakuntala (2000). Furthermore, (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one, curcumenol, benzofuran,6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-,trans-, cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1α,2β,4β)]-, curdione, and reynosin were newly identified as major components in the essential oil of this species. Nonetheless, curdione was reported as a major component in *Curcuma aromatica* (Angel et al., 2014) and *Curcuna wenyujin* (Zhang et al., 2017). While previous investigations by Pandey and Chowdhury (2003) and Paw et al. (2019) reported camphor, 1,8-Cineole, and epicurzerenone as predominant components. Lately, similar researches have been conducted on the essential oil of *C. caesia* and highlighted camphor, 1,8-cineole, curzerenone, and α-bulnesene as major compounds (Benya et al., 2023; Gangal et al., 2023; Mahanta et al., 2022). Our findings align with these investigations to a certain extent, yet curzerenone and α-bulnesene were absent in our studies. However, there is considerable quantitative variation in the percentage of the compounds identified.

Geographical variations in the chemical profiles were further underscored by comparisons with studies from other regions. Tropolone was noted to be the predominant component in the essential oil of this species from South India (Mukunthan et al., 2014), while Cycloisolongifolene,8,9-dehydro-9-formyl was predominant in North India (Kumar et al., 2020). Similarly, androsta-1,4-dien-3-one,17- (acetyloxy)-, (17.beta.) santanol acetate, eucalyptol, and cycloprop[e]indene-1a,2(1H) dicarboxaldehyde,3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl, (1a. alpha., 3a. beta., 6a. beta., 6b. alpha) were reported as major constituents from Arunachal Pradesh (Borah et al., 2020). Interestingly, the present findings differed significantly from these studies, except for eucalyptol, epicurzerenone, and camphor. Additionally, the findings of the species from Thailand were compared with our findings, where 1,8-cineole, camphor, curzerene, and curzerenone were reported as major compounds (Buddhasukh et al., 1995). 1,8-cineole and camphor are in congruence with their findings; however, curzerene and curzerenone were not identified in the study. Hence, the species grown in Manipur is deficient in curzerene and curzerenone.

Table 2: Chemical profile of rhizome essential oil of four *C. caesia* accessions from Manipur, India.

SL.No.	**Compound**	**RT**	**Area %**			
			CMP005	**CMP006**	**CMP009**	**CMP025**
1	Camphene	6.11	0.84	-	1.86	-

2	β-Pinene	6.99	2.24	2.32	-	-
3	Eucalyptol	9.02	**8.35**	3.38	**8.78**	3.4
4	1-Methylcyclooctanol	11.15	1.67	-	-	-
5	2-Nonanone	11.18	-	1.99	-	-
6	Cyclohexane,1,2,3-trimethyl-	11.88	-	**4.07**	-	-
7	Camphor	13.58	**4.59**	-	**7.97**	2.43
8	Isoborneol	14.08	1.47	-	1.7	-
9	2-Dodecanone	15.33	-	0.59	-	-
10	L-α-Terpineol	15.51	2.24	-	1.08	-
11	2-Cyclohexen-1-ol,2-methyl-5-(1-methylethenyl)-,cis-	17.04	0.43	-	-	-
12	Bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethyl-,acetate,(1S-endo)-	19.53	2.43	-	-	-
13	2-Undecanone	19.67	-	0.76	-	-
14	trans-Pinocarvyl acetate	19.96	0.18	-	-	-
15	Cyclohexene,4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-(3R-trans)-	21.67	2.17	2.78	1.39	-
16	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1α,2β,4β)]-	24.28	**6.45**	**10.85**	**4.48**	**6.58**
17	Tricyclo[4.4.0.0(2,7)]decane,1-methyl-3-methylene-8-(1-methylethyl)-,stereoisomer	25.12	2.04	-	-	-
18	Caryophyllene	25.18	-	**4.4**	-	-
19	γ –Elemene	25.76	3.73	**6.65**	2.93	3.22
20	Humulene	26.66	-	**5**	-	-
21	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo [4.4.0.02.7]decane-rel-	27.51	-	2.31	-	-
22	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo [4.4.0.02.7]decane-rel-	27.56	0.48	-	-	-

23	Tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene,trans-2,9-anti-9,10-trans-1,10-	28.51	-	**10.39**	-	-
24	Benzofuran,6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-,trans-	28.512	**6.58**	-	**8.30**	**9.84**
25	Aromadendrene oxide-(2)	29.213	**-**	-	0.96	-
26	1,5-Cyclodecadiene,1,5-dimethyl-8-(1-methylethylidene)-,(E,E)-	29.51	1.24	-	-	-
27	Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,[1R-(1α,3aβ,4α,7β)]-	29.52	-	1.59	-	-
28	Isoaromadendrene epoxide	30.49	-	0.91	-	-
29	Santalol,cis,α-	30.83	0.43	-	-	-
30	(-)-Globulol	31.70	1.06		0.66	
31	(R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one	32.78	**9.74**	**9.34**	**7.69**	-
32	Epicurzerenone	33.90	**11.22**	-	-	**41.44**
33	Curcumenol	34.67	**7.04**	-	**6.60**	**9.84**
34	(-)-Spathulenol	34.71	**-**	-	**-**	0.67
35	Valerenic acid	35.06	3.44	-	2.27	-
36	7a-Isopropenyl-4,5-dimethyloctahydroindene-4-carboxylic acid	35.23	-	2.18	-	-
37	(1aR,4aS,8aS)-4a,8,8-trimethyl-1,1a,4,4a,5,6,7,8-octahydrocyclopropa[d]naphthalene-2-carbaldehyde	35.66	-	-	1.86	-
38	Squamulosone	36.11	0.35	-	-	-
39	3,5,8a-Trimethyl-4,4a,8a,9-tetrahydronaphtho[2,3-b]furan	36.66	-	-	**5.07**	-
40	4,4´-Dimethyl-2,2´-dimethylenebicyclohexyl-3,3´-diene	37.03	**4.2**	-	-	-
41	Reynosin	37.55	-	-	-	**8.7**
42	Curdione	37.8	-	**12.7**	-	-

		3				
43	Acetic acid,6-(1-hydroxymethyl-vinyl)-4,8a-dimethyl-3-oxo-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester	38.47	3.21	-	-	-
44	5,8-Dihydroxy-4a-methyl-4,4a,4b,5,6,7,8,8a,9,10-decahydro-2(3H)-phenanthrenone	39.45	2.68	-	**16.14**	-
45	β-Cyclocostunolide	40.02	**4.99**	-	**9.46**	-
46	Furanodienone	40.13	-	0.91	-	1.63
47	Curcumenone	41.23	-	3.38	-	2.14
48	(4aR,5S)-1-Hydroxy-4a,5-dimethyl-3-(propan-2-ylidene)-4,4a,5, 6-tetrahydronaphthalen-2(3H)-one	41.71	-	-	**4.47**	-
49	2-Isopropenyl-2,3-dihydrofuro[3,2-g]chromen-7-one	45.25	0.88	-	-	-
50	Zederone	45.80	0.62	0.54	-	-
	Total compounds identified(%)		**96.99**	**87.04**	**93.67**	**89.89**

RT= Retention Time

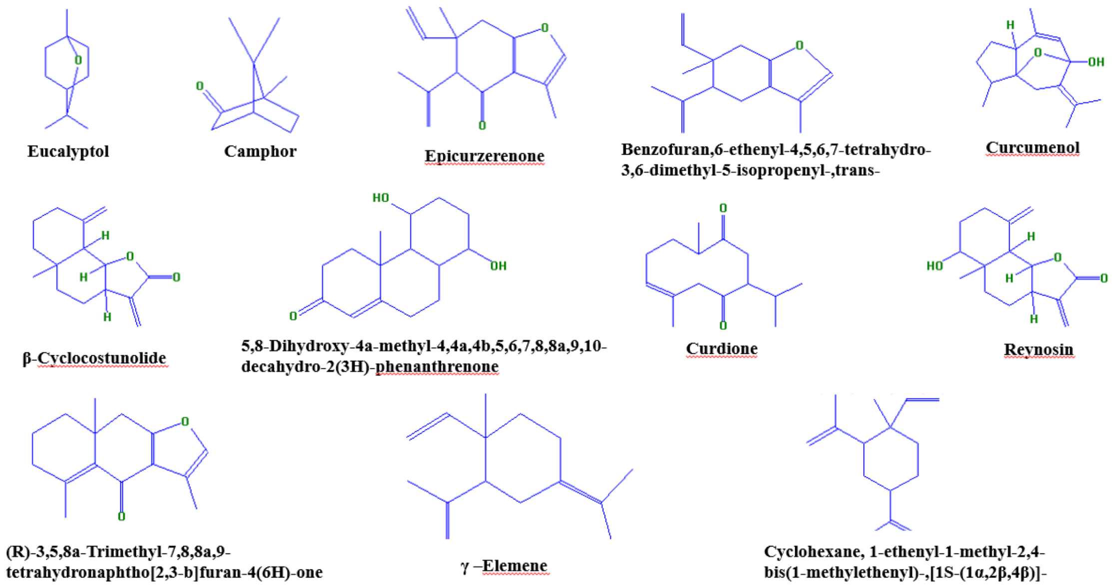

Figure 2: Molecular structures of major components of *C. caesia*.

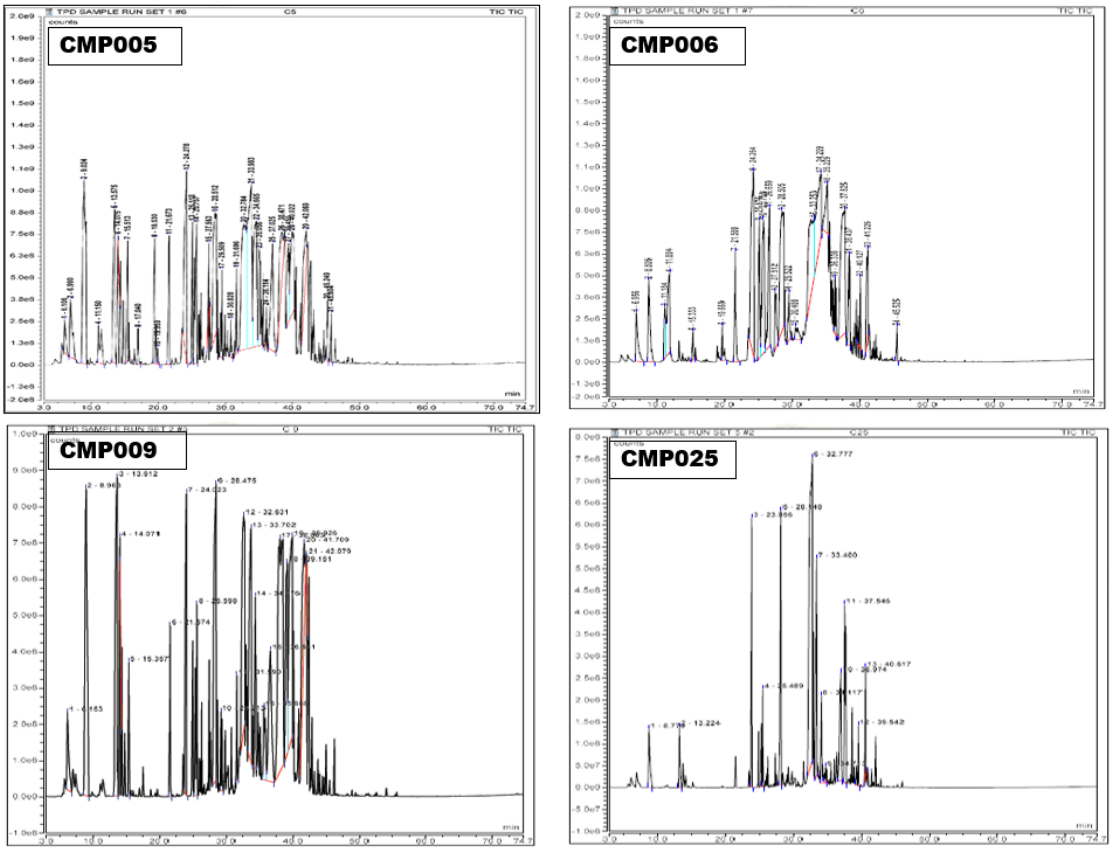

Figure 3: GC/MS Chromatograms of four accessions of *C. caesia* rhizome essential oil.

4. Conclusion

The present investigation highlights the variability in the chemical components of the rhizome essential oil, with major compounds including epicurzerenone, (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one, eucalyptol, curdione, and curcumenol. These findings offer valuable insights into the chemical profile of the volatile oils of *C. caesia*, which will pave the way for novel drug development and herbal remedies utilising this indigenous species.

Credit authorship contribution statement

Takhelmayum Priyadini Devi: Writing – original draft, Methodology, Data curation, Validation, Conceptualization. **Bedabati Chowdhury:** Writing – review & editing, resources, Supervision. **Md Aminul Islam**: Writing – review & editing, Conceptualization, Supervision. **Sudarshana Borah:** Writing – review & editing, Validation.

Declaration of Competing Interest

The authors declare no conflict of interest.

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