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Analysis of volatile components from inflorescence and rhizome of *Curcuma angustifolia* Roxb. by GC-MS.

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Abstract

Curcuma angustifolia Roxb., a rhizomatous, endangered medicinal and aromatic plant species, belongs to the family Zingiberaceae. The essential oil extracted from the inflorescence and rhizome by using methanol was characterised by GC-MS. The present investigation found that the total oil yield (% v/w) of the inflorescence and rhizome was 6.81 ± 0.80% and 1.45 ± 0.90%, respectively. The GC-MS investigation revealed the presence of 18 identified constituents, comprising 64% oil in the inflorescence, while 14 compounds were identified from the rhizome, containing 77% oil. The floral extract constituted with compounds mostly 2,2,7,7-tetramethyl-tricycle [6.2.1.0(1,6)]-undeca-4-en-3-one (15.635%), followed by β-caryophyllene (2.190%), isocericenin (0.782%), methyl isotridecanoate (0.724%), germacrene D (0.674%), *etc*., while the main components in the rhizome oil were germacrol (27.519%) followed by ß-pinene (16.460%), β-elemene (15.321%), β-elemenone (8.432%), germacrene (5.681%), 10-chlorotricyclo[4.2.1.1(2,5)]-deca-3,7-dion-9-ol (3.21%), *etc*. The presence of various secondary metabolite compounds, with a majority of sesquiterpenes in the inflorescence and rhizome of *C. angustifolia*, can be utilised as pharmacognostical tools for the preparation of drugs.

1. Introduction

Curcuma angustifolia Roxb., a prized edible plant among the Manipuris, fondly known as *Yaipal*, belongs to the genus *Curcuma* in the family Zingiberaceae. This species is found to commonly found growing in the wild in the Indian subcontinent, mainly in the Northeastern Region, the Western Coastal Plain and the sub-mountainous regions. Occurrence of this plant is reported from neighbouring countries, *viz.,* Burma, Laos, Nepal, and Pakistan (Ravindran *et al.*, 2007). *C. angustifolia* is a rhizomatous herb producing pink inflorescence during the spring season (March to April). Rhizomes of *C. angustifolia* are stout and grow up to 4.9 ft in length. The medicinal values are obtained from the rhizome of this plant, mostly, as it functions as the main nutrient storage site for this genus and is widely incorporated in Ayurvedic medicines and traditional treatments for many ailments (Phurailatpam *et al.,* 2024).

In Manipur, the floral part of *C. angustifolia* is consumed for its distinct pleasant aroma that complements well with any preparation method, either cooked or boiled. It is a culturally important delicacy for the Manipuris during the festive season of *Cheiraoba* (Meitei New Year), especially celebrated for its *Eromba* chutney. Not only the rhizome, but its leaf is also a source for oil that carries strong antimicrobial properties (Shukla *et al*., 2011), and the essential oil extracted from this species has been used for its antibacterial and antifungal properties (Doble *et al*., 2011). Both leaves and rhizome of *C. angustifolia* have a unique aroma resembling that of camphor and diverse functional constituents such as phenolics, flavonoids and various antioxidative enzymes (Jena *et al*., 2016). Traditionally, the rhizome of this plant is used for curing peptic ulcers, diarrhoea and colitis, and is also used in the treatment of dysentery (Patel *et al*., 2015). Consumption of *C. angustifolia* flower during the tender stage of inflorescence boosts the immune system (Assumi *et al*., 2017). Starch obtained from the rhizome possesses great medicinal value and is used in the preparation of many medicines which have great effectiveness for many diseases. The plants are also known to have antioxidant (Nahak and Sahu, 2011), anti-proliferative (Assumi *et al*., 2017), anti-cancerous (Nayak *et al*., 2013), antimicrobial (Jadhao and Bhuktar, 2017), anti-ulcerogenic (Rajashekhara *et al*., 2014), and antidiabetic activity (Sheikh *et al*., 2015).

2. Materials and Methods

2.1 Collection of samples

Plant sample parts as rhizomes and inflorescences of *C. angustifolia,* were collected from the ICAR Research Farm, Langol, Imphal West,

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Manipur, which lies within the latitude of 24°50'25"N and longitude of 93°55'35"E and at an elevation of 828 msl. The collection site is depicted in Figure 1. The natural population of *C. angustifolia* is predominantly linked with the hilly regions. The plant identity was confirmed based on assessment against the authenticated specimen of *Curcuma angustifolia* Roxb. by Ningombam *et al.* (2014) bearing Voucher Number MUMS 31599 in the deposited herbarium of Manipur University, Canchipur, Manipur, India.

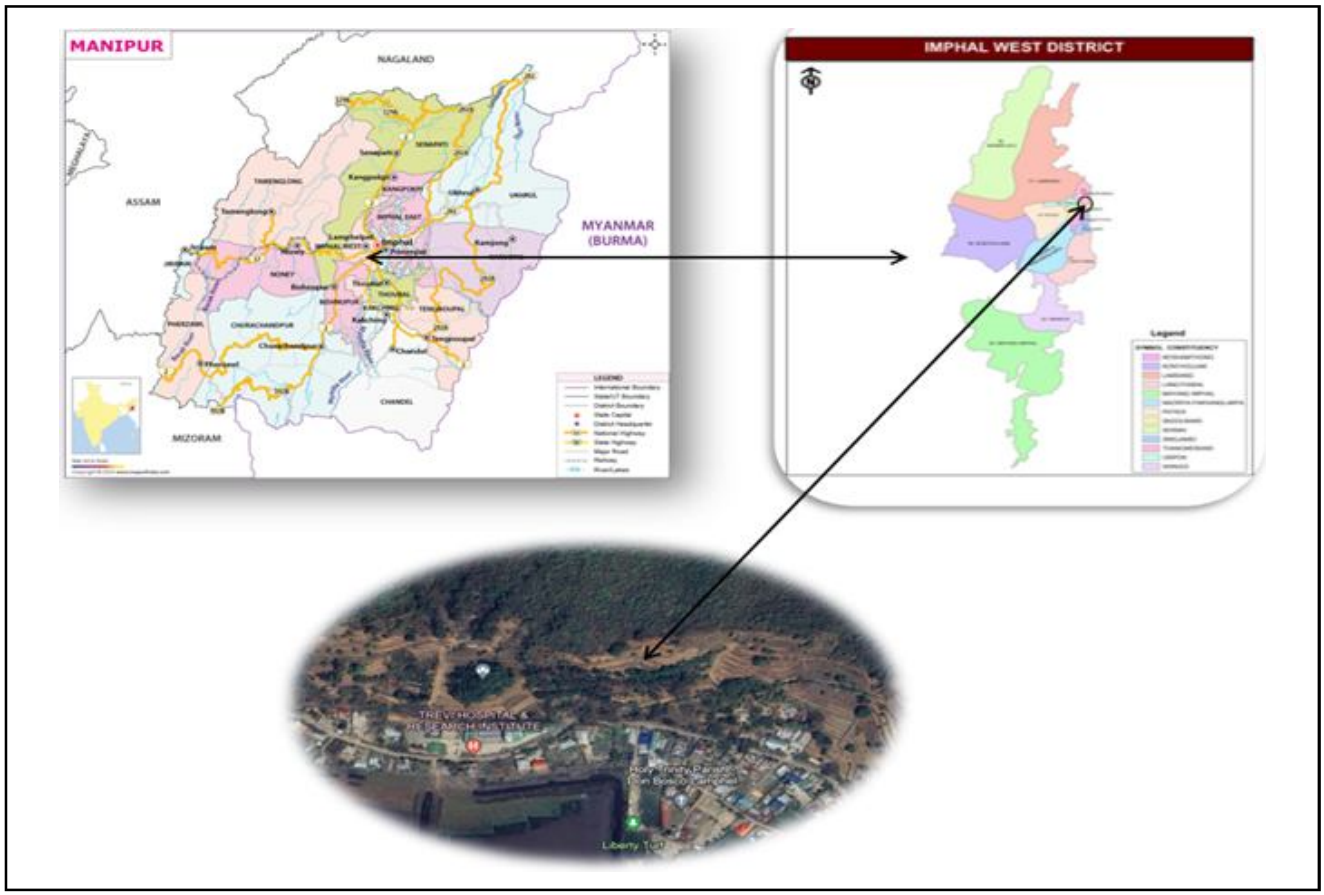

Figure 1: Geographical map showing the collection sites of *C. angustifolia*, ICAR Research farm, Langol, Imphal West, Manipur.

2.2 Plant materials and sample preparation

The fresh inflorescences that showed full pink colour were harvested from the field. Approximately 250 g each of fresh inflorescence (Figure 2a) and fresh rhizome (Figure 3a) were collected and then oven-dried at a temperature of 45°C for three days (Figure 2b and 3b).

Figure 2a: Fresh *C. angustifolia* inflorescence.

Figure 2b: Dried *C. angustifolia* inflorescence.

Figure 3a: Fresh *C. angustifolia* rhizomes.

Figure 3b: Dried *C. angustifolia* rhizomes.

2.3 Essential oil extraction

The dried samples were extracted using the Soxhlet apparatus with methanol as the solvent. Oil extractions were carried out at ICAR for the NEH Region, Lamphelpat. Here, the powdered plant samples were placed in a thimble inside the apparatus, and then the solvent (methanol) was heated in a flask at the bottom. The solvent then vaporised and condensed in a condenser. The condensed solvent then drips onto the sample, dissolving the oil. Later, the solvent-oil mixture was siphoned back into the flask, and the extraction cycle was repeated continuously to get the essential oil. The oil samples were dried over anhydrous Na_2SO_4 and stored in a sealed vial at 4°C until further analysis. Reagents comprised of deionised water, methanol, GC-MS grade, Merck (Darmstadt, Germany) and helium as carrier gas were used for oil extraction.

The oil yield was calculated using the formula:

$$\text{Percentage oil yield} = \frac{\text{weight of oil (g)}}{\text{weight of sample (g)}} \times 100$$

*Triplicate distillations were performed for each sample.

2.4 GC-MS analysis

GC-MS analysis was done following the User's Guide of Clarus 600/680 GC (PerkinElmer Life and Analytical Sciences, 2010) and the data were generated accordingly.

2.5 Compound identification

Compounds were identified by comparing the retention time (RT) and mass spectra (MS) data attained from the NIST library with the literature data (Adams, 2007). Retention indices of every component with the aid of a homologous n-alkane series (C8- C20) under the same GC section for every component found in the rhizome and inflorescence samples were determined by integrating peak area with an analysis program.

3. Results

3.1 Flower volatile oils

Essential oil of *C. angustifolia* flower was obtained by using the Soxhlet apparatus. The extracts were yellowish brown in colour. The results revealed that the samples were a good source of essential oils. The yield of essential oil (% V/W) of the flower samples was 6.81%, which is presented in Table 1.

3.2 Rhizome volatile oils

Volatile oils were extracted from the rhizomes of *C. angustifolia* using the hydro-distillation method, yielding 1.45% (v/w) essential oil (Table 1). The extracted oil was subsequently analysed using gas chromatography-mass spectrometry (GC-MS) to determine its chemical composition. A total of 14 compounds comprised 77% oil (Table 3, Figure 5). Major constituents found in the rhizome were germacrol (27.519%) followed by β-pinene (16.460%), γ-elemene (15.321%), β-elemenone (8.423%), germacrene (5.681%), 10-chlorotricyclo[4.2.1.1(2,5)]-deca-3,7-dion-9-ol (3.21%), retinene (2.293%), caryophyllene (2.486%), β-patchoulane (1.544%), β-elemene (1.301%), *etc.*

Table 1: Yield (%) of essential oil from *C. angustifolia* inflorescence

Collection site	Colour	Yield (% $^{V}/_{W}$)	
		Flower	Rhizome
ICAR Research Farm, Langol Hill	Deep brown	6.81 ± 0.80	1.45 ± 0.90

The identified compounds represented a good oil content in the flower (73%). The chromatogram is represented in Figure 4. GC-MS identified a total of about 18 compounds comprising 64% oil in the inflorescence. The floral extract constituted mainly of 2,2,7,7-tetramethyl-tricyclo-[6.2.1.0(1,6)]-undec-4-en-3-one(15.635%) and other compounds. Sesquiterpenes such as β-caryophyllene (2.190%), β-germacrene (0.636%), α-caryophyllene/humulene (0.456%), γ-elemene (0.275%) and germacrene D (0.674) were identified from the inflorescence, having pharmaceutical uses.

Table 2: Chemical composition, their retention time, molecular weight and relative peak area (%) of methanolic extract of *C. angustifolia* flower extracts

S. No.	Compound	Retention time	Molecular weight	Relative peak area (%)
1	β- phellandrene	12.870	136	Trace
2	Eucalyptol	15.191	154	0.121
3	Camphore	20.323	152	0.254
4	β- germacrene	25.135	204	0.636
5	Cis-chrysanthemol	25.200	376	Trace
6	β-caryophyllene	26.336	204	2.190
7	Ò-elemene	26.426	204	0.257
8	β-farnesen	26.571	204	Trace
9	α-caryophyllene	27.366	204	0.456
10	Germacrene D	28.116	204	0.674
11	α-funebrene	28.446	204	0.367
12	Isocericenin	29.077	260	0.782
13	β-elemenone	32.373	218	0.403
14	Bicyclo-(3.3.0)-oct-1-en-3-one	33.598	122	0.391
15	Methyl isotridecanoate	38.035	228	0.724
16	Butyl lauryl phthalate	42.082	390	Trace
17	Methyl-9-cis-11-trans-octadecadienoate	42.297	294	Trace
18	2,2,7,7-tetramethyl-tricycle-[6.2.1.0(1,6)] -undeca-4-en-3-one	44.573	218	15.635

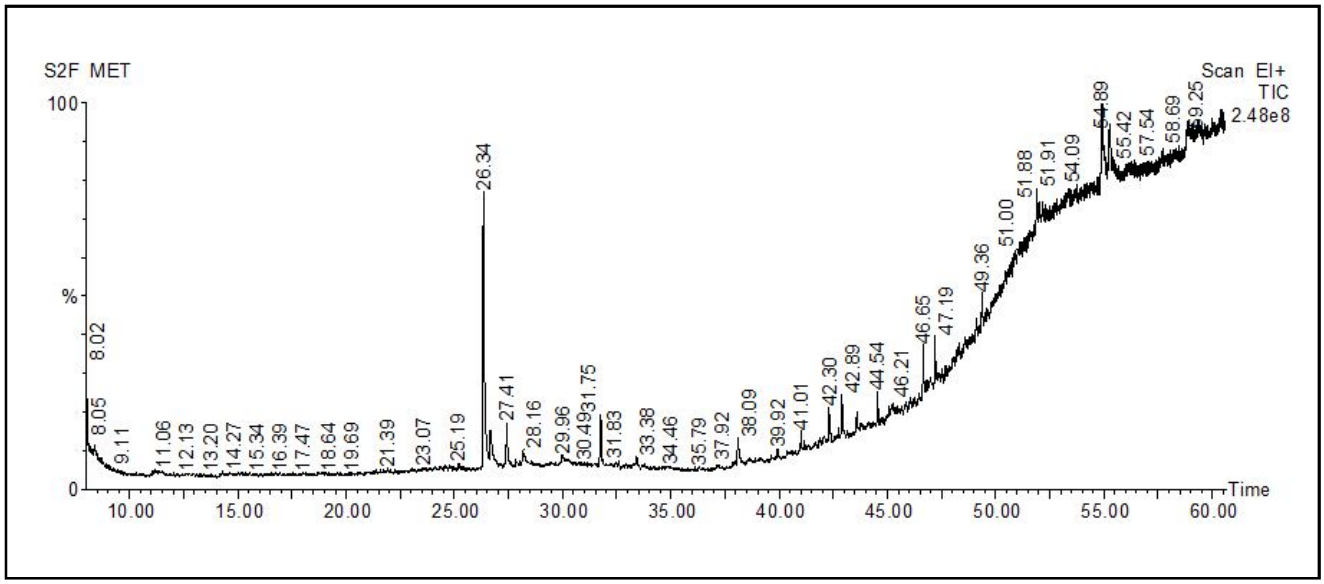

Figure 4: GC-MS chromatogram of volatile oil obtained from *C. angustifolia* flower.

3.3 Rhizome volatile oils

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Table 3: Chemical composition, their retention time, molecular weight and relative peak area (%) of methanolic extract of *C. angustifolia rhizome* extracts

S.No.	Compound	Retention time	Molecular weight	Relative peak area (%)
1	β-elemene	25.780	204	1.301
2	β-elemenone	33.478	218	8.423
3	Germacrene	33.754	218	5.681
4	β-pinene	34.879	180	16.460
5	γ-elemene	35.134	204	15.321
6	β-patchoulane	35.194	206	1.544
7	Caryophyllene	38.570	204	2.486
8	Germacrol	41.487	190	27.519
9	α-guariene	44.748	204	0.594
10	10-chlorotricyclo[4.2.1.1(2,5)] -deca-3,7-dion-9-ol	45.303	182	3.21
11	Retinene	49.150	284	2.293
12	Isoaromadendrene	50.750	204	0.795
13	Methyl acetoacetate	50.955	390	0.766
14	Trans- longipinocarveol	51.095	220	0.754

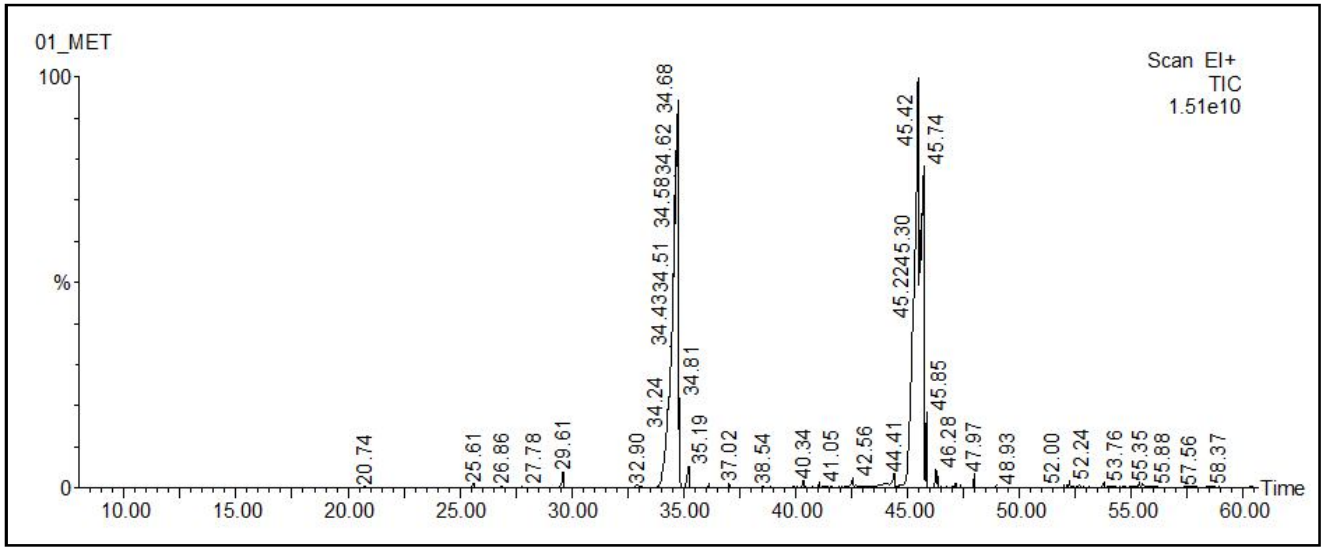

Figure 5: GC-MS chromatogram of volatile oil obtained from *C. angustifolia* rhizome.

4. Discussion

In India, *C. angustifolia* has been traditionally used in indigenous medicine for treating numerous diseases (Sharma *et al*., 2019). However, the essential oil from the inflorescence of *Curcuma* sp. has not yet been exploited much, while numerous studies have been carried out on rhizomes. Flower oil of *C. angustifolia* contains b-germacrene, 2,2,7,7-tetramethyl-tricycle[6.2.1.0](1,6)]undeca-4-en-3-one, caryophyllene oxide, b-caryophyllene, methyl-isomyristate, and germacrone as the major constituents (Devi *et al.*, 2021), which is in corroborate with the result of our findings. Comparison of volatile oils constituents of *C. angustifolia* rhizomes of central and southern India was conducted by Srivastava *et al.* (2006), where they found that central Indian rhizomes contain high amounts of xanthorrhizol isomer, methyl eugenol, palmitic acid, and camphor, while the southern Indian rhizomes mainly consisted of camphor, germacrone, isoborneol, curdione, borneol, and 1,8-cineole. Devi *et al.* (2021) identified a total of 24 compounds from the rhizome, amongst which the major constituents comprise germacrone, α-pinene, α-elemene, α-elemenone, germacrene, and caryophyllene. However, Murthy *et al*. (2024) found that the rhizome oils consisted mainly of r-turmerone, benzene butanal, bornyl acetate, turmeronol, (E)-atlantone, eucalyptol, 14-hydroxycaryophyllene, and 5-azulenemethanol, which is totally in contrast to the present investigation. In *Zingiber officinale* and *Kampaferia parviflora*, the major volatile compounds found in rhizomes are α-terpineol, borneol,

linalool, α-copaene, g-elemene, and β-sesquiphellandrene (Vaishnavi *et al*., 2024), while the main constituents in the present findings are Germacrol, β-pinene, and g-elemene as the major rhizome oil constituents. This reveals that g-elemene is one of the major oil constituents in rhizomes of the plant family Zingiberaceae. Quantitative and qualitative variations of oil in the plant could be due to varying geographical locations of the species (Vaishnavi *et al*., 2024), ecological factors (Raut and Karuppayil, 2014), genetic variation (Sangwan *et al*., 2001), stress (Rahimmalek *et al*., 2013), and genetic pattern (Hassiotis *et al*., 2014).

The major oil constituent found in the inflorescence, *i.e.,* 2,2,7,7-tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one, has anti-cancerous properties (Taunk *et al*., 2018). The β-caryophyllene obtained from the inflorescence is known to exhibit antibacterial, antioxidant, gastroprotective, anxiolytic (Gupta and Phulara, 2021) and anti-inflammatory properties (Freire *et al*., 2014); anti-ageing (Cheng *et al*., 2014) and neuroprotective (Pant *et al*., 2014).

The major constituent of rhizome oil (germacrone) has been shown to improve liver fibrosis (Ji *et al*., 2021); inhibit the progression of lung cancer (Zhao *et al*., 2021); and Prostate cancer (Yu *et al*., 2020). It also showed anti-inflammatory properties (Hossain *et al*., 2015); antiandrogenic (Suphrom et *al*., 2012); antimicrobial (Kamazeri *et al*., 2012) and antiviral (Riaz *et al*., 2020) activities. g-elemene has been shown to have potent anticancer properties and has been used frequently in Chinese medicine. It inhibits the growth of tumours and induces apoptosis in cancer cells. (Tan *et al.,* 2021) and also exhibit antileishmanial activity (Nunes *et al*., 2021)

5. Conclusion

The current study proved that methanol extraction gave many bioactive constituents with extensive pharmacological and biological activities. The compounds have great prospects for the formulation of therapeutic agents through conventional and contemporary medicinal practices. The research presented useful information on the volatile oil composition and methanolic extract of *C. angustifolia* harvested from Manipur. GC-MS analysis established 18 compounds in the flower oil and 14 compounds in the rhizome oil, largely sesquiterpenes, as established by the richness of secondary metabolites. These compounds could be valuable markers of pharmacognosy. The results indicate that the methanolic extract of this plant shows great therapeutic promise. The present work provides the basis for designing new treatment regimens based on plant extracts. Nevertheless, it will be important to conduct more studies to determine, isolate, and establish the purity of the active components causing the noted therapeutic responses.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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