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Research Article

Phenotypic and biochemical characterization of Turmeric (*Curcuma longa* L.) during developmental stages.

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Abstract

Turmeric is noted for its rhizomes' significant accumulation of secondary active metabolites and other bioactive compounds. This study aims to delve into the intricate biochemical mechanisms that govern the growth and development of turmeric. It investigates patterns and variations in biochemical processes across different developmental stages. Usually, turmeric crops are harvested between the 7th and 9th months. Therefore, the experiment was designed to collect rhizomes every 3rd, 6th and 9th months. Morphological observations were recorded during every three-month interval. The current study centers on turmeric plants' phenotypical and biochemical characteristics at the 3rd, 6th and 9th month developmental stages. Notably, plant height consistently increased across all stages, with the highest recorded height observed in 9-month-old plants. Within the canopy, both leaf length and leaf width displayed a gradual augmentation throughout the monitoring period. The 9-month-old plants demonstrated the highest leaf numbers. Rhizome yield exhibited an ascending trend over time, with weights of 221g, 623g and 929g recorded in the 3rd, 6th, and 9th months, respectively. The curcumin content was 2.13%, 3.30 %, and 4.47 % in the 3rd, 6th and 9th months, respectively; likewise, the protein content was 5.76 ± 0.9 %, 3.11 ± 1.0 %, and 6.34 ± 0.8 % in the rhizomes of *C. longa* at the 3rd, 6th and 9th month growth stages, respectively. Essential oil of rhizomes GC-MS data revealed that 40 major compounds were shared: turmerone (26.5 %), ar-turmerone (25.4 %), curlone (15.8 %), alpha-phellandrene (5.09 %), and eucalyptol (2.76 %). In addition, a comprehensive biochemical analysis of *C. longa* rhizomes including the integration of color analysis, pH measurement, assessment of proteins, determination of total flavonoids and phenolics, and evaluation of FRAP and DPPH antioxidant activitywere carried out.

1. Introduction

Turmeric (*Curcuma longa* L.) is a spice crop belonging to the Zingiberaceae family, thriving in tropical regions, primarily Southeast Asia. Cultivated for its rhizome, characterized by rough segmented skins. The rhizome can grow to a length of 2.5– 7.0 cm with a diameter of 2.5 cm [1]. The rhizome has a balmy smell and bitter taste. *C. longa* plants are grown in tropical and subtropical regions at around 20 °C and 30 °C with sufficient rainfall [2]. The plant is renowned for its culinary and medicinal significance, turmeric extends its influence beyond food, serving diverse roles in the cosmetic and pharmaceutical industries as well as enhancing culinary experiences as a food additive and flavoring agent [3]. India is the major producer and exporter of turmeric accounting for approximately 80 % of total production [4]. Its

ground rhizomes have important medicinal components like curcuminoids, flavonoids, phenolic acids, and essential oil, which explain its worldwide recognition as a functional food [5]. The rhizomes contain a spectrum of active ingredients with multifaceted properties, including antioxidant, antiarthritic, antimutagenic, antitumor, antithrombotic, antivenom, antibacterial, antifungal, antiviral, nematocidal, choleretic, antihepatotoxic, and hemagglutinating activities [6, 7]. The major classes of secondary metabolites in turmeric are polyphenolic curcuminoids containing a mixture of curcumin (60–80 %), demethoxycurcumin (15–30 %), and bisdemethoxycurcumin (2-6 %) [8]. Turmeric is harvested when the aerial part of this plant's senescence and its rhizomes develop bright yellow to orange-yellow color. The characteristic yellowish color is due to the presence of polyphenolic pigment curcuminoids which make up 2–5 % of the rhizome mass [9]. The rhizome is soluble in methanol, ethanol, or dimethyl sulfoxide [10]. The quantification of curcuminoids, through spectrophotometric methods and high-performance thin-layer chromatography (HPLC) addresses the limitations associated with color-based spectrophotometric techniques [11]. Curcumin enhances its antioxidant activity by scavenging diverse reactive oxygen species, including superoxide radicals, hydrogen peroxide, and nitric oxide radicals. Additionally, it inhibits lipid peroxidation to further contribute to its antioxidant capabilities [12]. Antioxidants are an important part of our regular diet that prevents oxidative cell damage by acting as free radical scavengers [13]. Phenolics are major secondary metabolites, that are present in climacteric and non-climacteric fruit. An essential biological function of phenolic compounds is their antioxidant properties. Flavonoids are common secondary metabolites found in plants with a wide range of biological functions, including coloration, defense against biotic and abiotic stressors, and contributing to plant growth and development [14]. The largest and most prominent category of polyphenols, the flavonoids, and their derivatives offer potent anti-inflammatory, anticancer, hepatoprotective, and antioxidant action due to their capacity to scavenge reactive oxygen species and reduce oxidative stress [15]. In this context, the present investigation was undertaken to gain insights into the morpho-biochemical characteristics of *C. longa* in different growth and developmental stages with respect to economic traits.

2. Materials and methods

2.1 Plant material

The *Curcuma longa* var Erode local was collected from Salundi, Mysore District, Karnataka (11°43'26.4"N, 76°41'56.4"E). Rhizomes were cultivated in an experimental field and maintained in triplicate under biological conditions, with proper watering and environmental care. Harvesting of the rhizomes occurred in the 3rd, 6th, and 9th months. Subsequently, the rhizomes were stored at -80 ˚C for biochemical assays. Fresh rhizomes were utilized for color analysis and extraction.

2.2 Morphological observations

Morphological observations were recorded from each plant in triplicate for all the 3rd, 6th, and 9th months respectively. Plant height was recorded by measuring the tallest tiller of the plant from the base up to the leaf tip and canopy was also measured for all the plants. Leaf length was measured from the tip of the leaf to the point of origin of the petiole. The width was measured from the middle point of the leaf length from one side to the other. The numbers of leaves are also counted for each plant. All the leaf measurements were taken from randomly selected three leaves from each plant avoiding those towards the base and the tip. All these measurements were expressed in cm and the mean of three values formed the value of the treatment in respective replication. Harvesting of rhizomes was carried out after 3rd, 6th, and 9th months respectively. The fresh weight of rhizomes was recorded after cleaning the rhizomes and expressed in gm. The dried weight of the rhizome was taken for a 100 gm fresh sample, and all phenotypic characters were recorded in biological triplicate [16].

2.3 pH measurement and color analysis

The pH changes of rhizome powder were investigated through a systematic procedure. Initially, 0.5 grams of dry rhizome powder were precisely measured and mixed thoroughly with 5 mL of Milli-Q grade water. This mixture was left to stand for 2 h, during which occasional stirring was employed to ensure uniform dispersion of the rhizome powder in the aqueous solution. Following the designated period, the pH of the solution was measured using a pH meter [17]. The

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color of the rhizomes was measured by a color-measuring instrument (Konica Minolta CM-5) according to the method of Pathare et al., [18]. Color values such as L*, a*, and b* of the dried rhizome powders were recorded as triplicates. C*, a*, b*, and h* represent chroma, red/green, yellow/blue, and hue angles, respectively.

The values for chroma (C*) and hue angle (h*) were computed using the following formula.

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \qquad h^* = tan^{-1}\left(\frac{b^*}{a^*}\right)$$

2.4 Analysis of carbon, hydrogen and nitrogen

The ultimate analysis was performed using an elemental analyzer (Euro EA Elemental Analyzer). This technique determines the content of carbon, hydrogen, nitrogen, and sulfur present in a sample. The analytical method relies on the thorough and rapid oxidation of the sample through combustion with oxygen at an approximate temperature of 1020 °C. The resulting combustion products are conveyed by a gas carrier to a chromatographic column where separation occurs. A thermal conductivity detector generates the signal for each element, which is subsequently translated into a percentage content [19].

2.5 Estimation of protein

N/Protein analyzer (Thermal/Flash 2000) can be used for the determination of nitrogen concentration and the relative protein content in turmeric powder. The N/Protein analyzer is based on the Dumas method (Flash Dynamic Combustion). The 50 mg (only) samples are weighed in tin capsules, placed inside the autosampler at a preset time, and then dropped into an oxidation/reduction reactor kept at a temperature of 900 - 1000 °C. The exact amount of oxygen required for optimum combustion of the sample is delivered into the combustion reactor at a precise time. The reaction of oxygen with the Tin capsule at elevated temperature generates an exothermic reaction which raises the temperature to 1800 °C for a few seconds. At this high temperature, both organic and inorganic substances are converted into elemental gases which, after further reduction, are separated in a chromatographic column and finally detected by a highly sensitive thermal conductivity detector (TCD).

2.6 Sample extraction for biochemical assays.

250 mg rhizome powder was homogenized in 25 mL of HPLC grade absolute methanol (Merck) for continuous shaking for 72 h. The extract was centrifuged at 10000 rpm for 20 min. The extracts were filtered and stored in -80 ˚C for biochemical assays.

2.7 Estimation of total phenolics

The Folin-Ciocalteu technique was used to quantify the total phenolic content (TPC) of crude extracts. A known amount of extract (0.1 mL) was pipetted into a test tube, which was then filled with distilled water to a volume of 3 mL. The mixture was then incubated for 3 minutes with 0.5 mL of Folin-Ciocalteu reagent, followed by 2 mL of 20 % (w/v) Na_2CO_3 solution. The tube was vortexed and immersed in hot water for exactly one minute. After cooling the tube contents, the absorbance at 650 nm was measured. A previously plotted gallic acid standard graph was used to quantify each sample's phenolics. TPC was measured in gallic acid equivalents (GAE)/100g [20].

2.8 Estimation of total flavonoids

The total flavonoid content (TFC) of each crude extract (0.1 mL) was diluted to an optimum concentration with 100% methanol. The diluted sample was then combined with 1 mL of a 2 % (w/v) $AlCl_3$ methanolic solution. After 15 minutes at room temperature, the absorbance of the reaction mixture at 430 nm was measured using a double-beam spectrophotometer. TFC was calculated as mg of quercetin equivalent (QE)/g [21].

2.9 Antioxidants assay for DPPH free radical scavenging activity.

In individual test tubes, diverse dilutions of each extract ranging from 0.125 to 1.25 mg mL^{-1} were prepared. Subsequently, 39.4 mg L^{-1} of 2,2-diphenyl-1-picryl-hydrazine-hydrate (DPPH) in a methanolic solution was introduced into each test tube, bringing the total volume to 2 mL. The contents of the test tubes were thoroughly mixed and left to incubate for 15 minutes in darkness. Methanol was utilized as a blank in this process. Absorbance readings were then taken at 517 nm, and the results were expressed as a percentage of scavenging activity. The IC_{50} value was determined from the percentage inhibition plotted against the concentration of different sample extracts (BLOIS 1958).

The radical scavenging activity was quantified using the following formula:

DPPH-scavenging activity (%) = [(OD control – OD sample)/OD control] *100,

where OD represents optical density.

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2.10 HPLC analysis for curcumin

Each rhizome sample (250 mg) was macerated with 25 mL of high-performance liquid chromatography (HPLC) grade methanol (Merck) for continuous shaking for 72 h. The extract was filtered through a syringe filter (pore size 0.22 µm). 10 mg/mL concentration was used for the HPLC analysis to determine the Curcumin. The HPLC system **(**SHIMADZU Model DGU-20A5R**)** was used to process the data. The separation was achieved by a reverse-phase Nova-pack C-18 column (SHIMADZU). The mobile phase consisted of solvent A made of 0.1 % acetic acid (AA) and solvent B made from absolute acetonitrile (ACN); the flow rate was adjusted to 1ml /min, the column temperature was thermostatically controlled at 35°C and the injection volume was kept at 10 µL. Total analysis time per sample was fixed for 15 minutes. HPLC chromatograms were detected using a PDA detector at 425 nm. Each compound was identified by its retention time and by spiking with standards under the same conditions. For the estimation of curcuminoids; methanolic extracts were analyzed in HPLC using an isocratic solvent system composed of 60 (A): 40 (B) [22].

2.11 Extraction of essential oils and GC-MS analysis

To extract the essential oil, newly obtained from the rhizome of *C. longa*, materials were hydro-distilled for 3-5 h using a clevenger-style apparatus. After being extracted and refrigerated until analysis, sodium sulfate is added for water absorption in essential oil [23].

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of *C. longa* essential oil was conducted utilizing an Agilent Technologies, USA 7890B GC 5977A MSD System. Sample preparation involved dissolving 10 µL of essential oil in 990 µL of HPLC grade n-hexane, with n-hexane used as the blank in GC-MS analysis. Compound quantification was determined by analysing the peak area percentage of the chromatograms. The identification of major constituents in the essential oil was based on their distinctive fragmentation patterns [24].

2.12 Statistical analysis

The data in this study is presented as mean ± SD. Statistical analysis followed standardized procedures, and Analysis of Variance (ANOVA) was conducted according to the methodology outlined by Panse and Sukhatme [25]. The results of all experiments were analyzed using software such as MS Excel 2019, SPSS, GraphPad Prism 8, etc. Analysis of variance and testing for significant differences among means were performed through ANOVA. The differences between means were assessed using the Tukey test.

3. Results

3.1 Morphological characterization

The morphological characteristics of the plant were systematically observed at intervals in the 3rd, 6th, and 9th months. Notably, plant height consistently increased across all stages, with the highest recorded height observed in 9-month-old plants. Throughout the monitoring period, there was a gradual increase observed in both leaf length and leaf width within the canopy. The 9-month-old plants demonstrated the highest leaf numbers. Rhizome yield exhibited an ascending trend over time, with weights of 221 ± 23.47 g, 623 ± 33.50 g, and 929 ± 61.11 g recorded at the 3rd, 6th, and 9th months, respectively (Fig 4a). The dry weight analysis, conducted on 100 g samples, revealed the highest dry weight in 9-month-old plants, followed by 3-month-old and 6-month-old plants. A comprehensive presentation of the morphological data is provided in Fig. 1. These findings underscore the dynamic growth patterns and increasing yields observed in various aspects of the plant's development over the monitored time points.

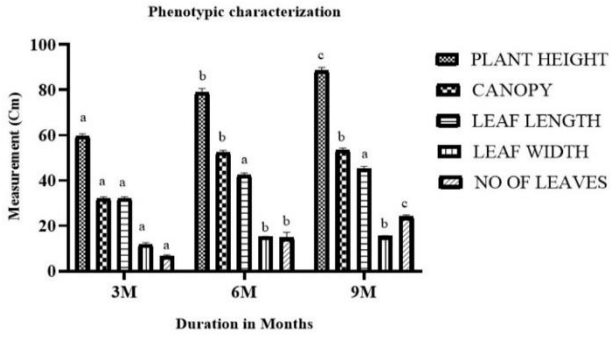

Figure 1. Phenotyphical characterization of plant height, Canopy, Leaf length, Leaf width and number of leaves values are represented as mean ± SD of three replicates, and the bars with different superscripts were significantly different from each other ($p < 0.05$).

3.2 pH measurement and color analysis

A systematic assessment of rhizome color was conducted, employing standardized color analysis techniques. The rhizome color varied during the developmental stage, the color was light yellow in the

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3rd month, and in the sixth months and 9th months harvest rhizome color was orange-yellow to dark yellow. The color parameters a* and b* were used to calculate the Chroma (C*), with higher Chroma (C*) indicating higher color intensity. The L* value signifying lightness in color was 55.48 ± 0.99, 65.91 ± 1.26, and 62.44 ±0 .01, and the C* value was 42.59 ± 4.46, 42.21 ± 2.64, and 53.20 ± 0.02. The hue (h°) values of the 3rd, 6th, and 9th months were 62.66 ± 0.03, 64.61 ± 0.02, and 71.60 ± 0.01 (Fig. 2). The rhizome pH during the 3rd, 6th, and 9th month was in the neutral range from 6.67, 7.92, and 6.23, respectively. The color content of the rhizome rules out the possibility of the involvement of pH in the observed change of color.

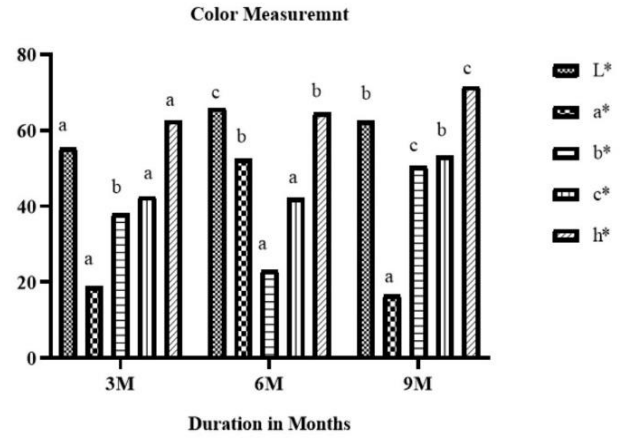

Figure 2. Color measurement during developmental stages, all of the values are represented as mean ± SD of three replicates. The bars with different superscripts for different duration of months were significantly different from each other ($p < 0.05$).

3.3 *Estimation of carbon, hydrogen and nitrogen*

The levels of carbon (C), hydrogen (H), nitrogen (N), and sulphur (S) percentages were analyzed using the rhizome samples collected during the 3rd, 6th, and 9th months of development. Nitrogen content was found to be the highest during the 9th month, followed by the 3rd and 6th months. Carbon content exhibited a different trend, with the highest levels observed in the 3rd month. This content gradually decreased by 1 % from the 3rd to the 6th month, followed by a further decline in the 9th month. Interestingly, hydrogen content remained consistent across all developmental stages. Overall, these findings suggest dynamic changes in the elemental composition of the rhizome samples over 9 months, with distinct patterns observed for nitrogen and carbon, while hydrogen content remained constant. (Fig. 3 and Fig. 3a).

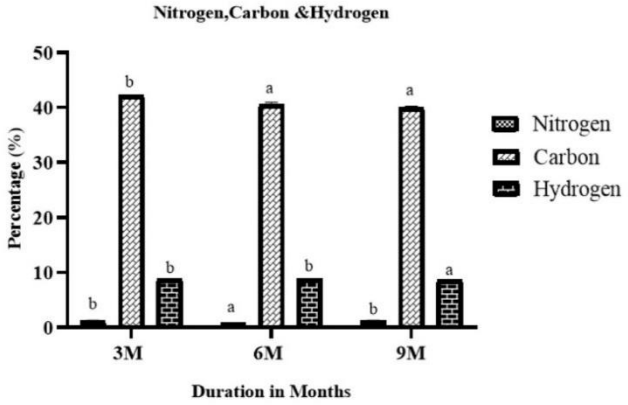

Figure 3. Nitogen, carbon and hydrogen pecrcenatge during 3rd, 6th and 9th months of *Curcuma longa*.

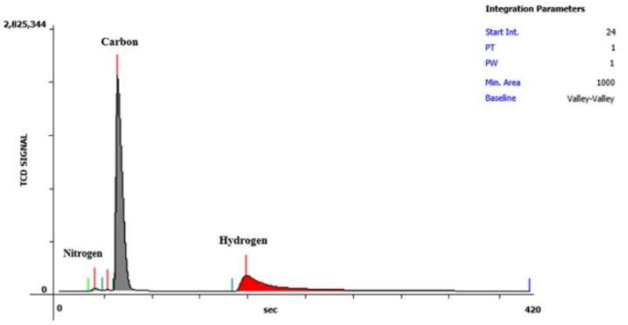

Figure 3a. C, H, N, S chromatogram of *Curcuma longa* during 9th month.

3.4 *Protein estimation*

Protein percentage levels were analyzed in rhizome samples collected during the 3rd, 6th, and 9th months of development in *C. longa*. The protein content was found to be 3.11 ± 1.0 %, 5.76 ± 0.9 %, and 6.34 ± 0.8 % in the rhizomes at the 3rd, 6th, and 9th-month developmental stages, respectively. The highest protein content was observed in the rhizomes in the 9th month. Rhizomes in the 3rd month exhibited a significantly lower protein content compared to those in the 6th and 9th months. These findings provide quantitative insights into the variation in protein levels during different stages of *C. longa* development, emphasizing the significance of temporal factors in influencing rhizome protein composition (Fig. 4e).

3.5 *Total phenolics and flavonoids*

During the developmental phases of *C. longa*, there was an increasing trend in the accumulation of phenolic and flavonoid compounds, reaching their peak during the mature stage. Total phenolic content (TPC) gradually increased in all stages. The results in the 9th month exhibited the highest TPC at 16.08±0.15

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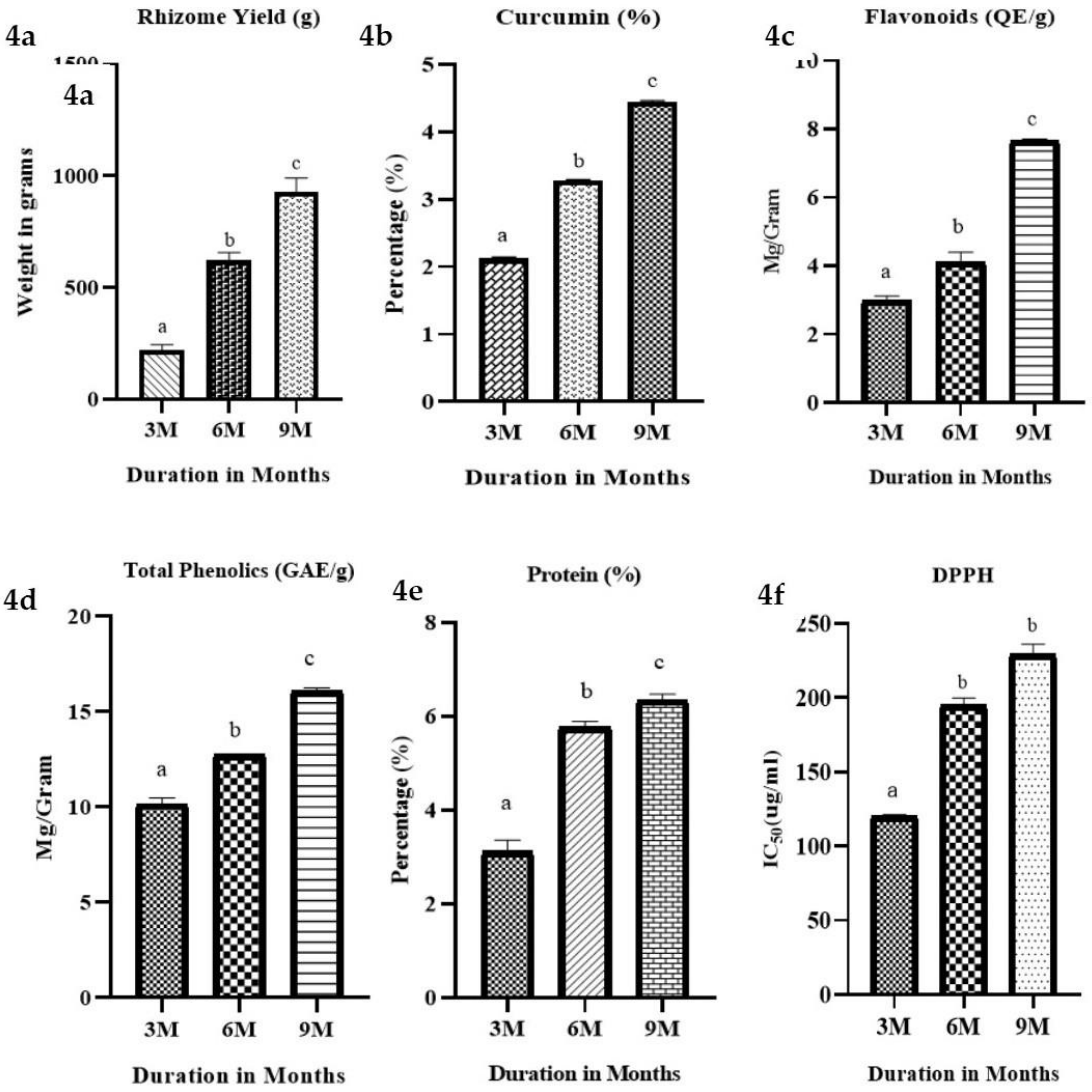

Figure 4. All of the values are represented as mean ± SD of three replicates. The bars with different superscripts represent 3rd, 6th and 9th months were significantly different from each other ($p < 0.05$). Rhizome yield (a), Curcumin (b), Flavonoids (c), Total phenolics (d), Protein (e) and DPPH (f).

mg GAE/g, followed by 12.73±0.02 in the 6th month, and 10.14±0.32 in the 3rd month. In the case of flavonoids, the highest concentration was observed in the 9th month (7.67±0.03), followed by the 6th month (2.98±0.14), and the 3rd month (4.11±0.29) (Fig. 4c, Fig. 4d).

3.6 Quantification of HPLC for curcumin

The HPLC analysis revealed a progressive increase in curcumin content from the 3rd to the 9th month in the rhizomes. The highest curcumin content, reaching 4.47%, was observed in the 9th month, while the 6th month exhibited 3.30%, and the 3rd month recorded 2.13% (Fig. 4b). Throughout the developmental stages, curcumin demonstrated a gradual increase of approximately 1%. Fig. 5 displays chromatograms for curcuminoids, including bisdemethoxycurcumin, dimethoxy curcumin, and curcumin.

3.7 GC-MS analysis for essential oil

GC-MS analysis identified and quantified the essential oil compounds, contributing to a comprehensive profile of the rhizome's essential oil chemical composition. The chemical characterization of *C. longa* essential oil constituted 40 compounds representing 98% oil composition identified. A representative gas chromatogram is shown in Fig. 6. In essential oil turmerone was the major compound ranging from 26.5%, followed by ar-tumerone (25.4%), curlene (15.8%), alphaphellan- drene (5.1%), eucalyptol (2.8%), tumerone (2.6%), benzene, 1-methyl-4-(1-methylpropyl)-(1.7%); tricyclo [2.2.1.0 (2,6)] heptane-3-methanol, 2,3-dimethyl- (1.5%); 6-(p-tolyl)-2-methyl-2-heptenol, trans-(1.4%); benzene,

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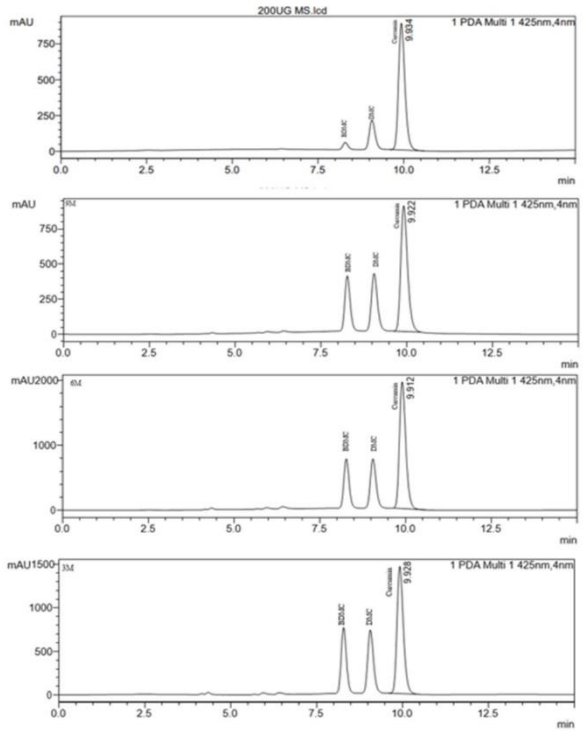

Figure 5. HPLC Chromatograms of bisdemethoxycurcumin, demethoxycurcumin and Curcumin during different developmental stages *in Curcuma longa*. Standard (S), 3rd Month (3M), 6th Month (6M) and 9th month (9M).

1,1'-(1,1,2,2-tetramethyl-1,2-ethanediyl) bis-(1.4%); 6-(p-Tolyl)-2-methyl-2-heptenol, trans- (1.3%) and other compound listed in Table 1.

3.8 DPPH (2,2-diphenylpicrylhydrazyl) Antioxidant assay for scavenging activity.

Antioxidant assays demonstrated a correlation between developmental stages and the rhizome's ability to scavenge free radicals, suggesting potential implications for its therapeutic efficacy**.** DPPH is a popular fast and repeatable approach for determining antioxidant activity in rhizome extracts. The antioxidant action is mostly attributed to phenolic and flavonoid chemicals, and the current findings are also related to their content. The DPPH scavenging activity assay shows inhibitory concentration (IC_{50}) values of 120.34± 0.915 mg mL^{-1}, 194.89 ± 5.007, and 229.33± 6.785 mg mL^{-1} in the 3rd, 6th, and 9th months, respectively (Fig 4f).

4. Discussion

Turmeric exhibits extensive phenotypic variability in traits such as leaf shape, leaf color, rhizome weight, rhizome color, aroma, and pungency. Surprisingly, none of these observed phenotypes have been conclusively linked to the medicinal properties of

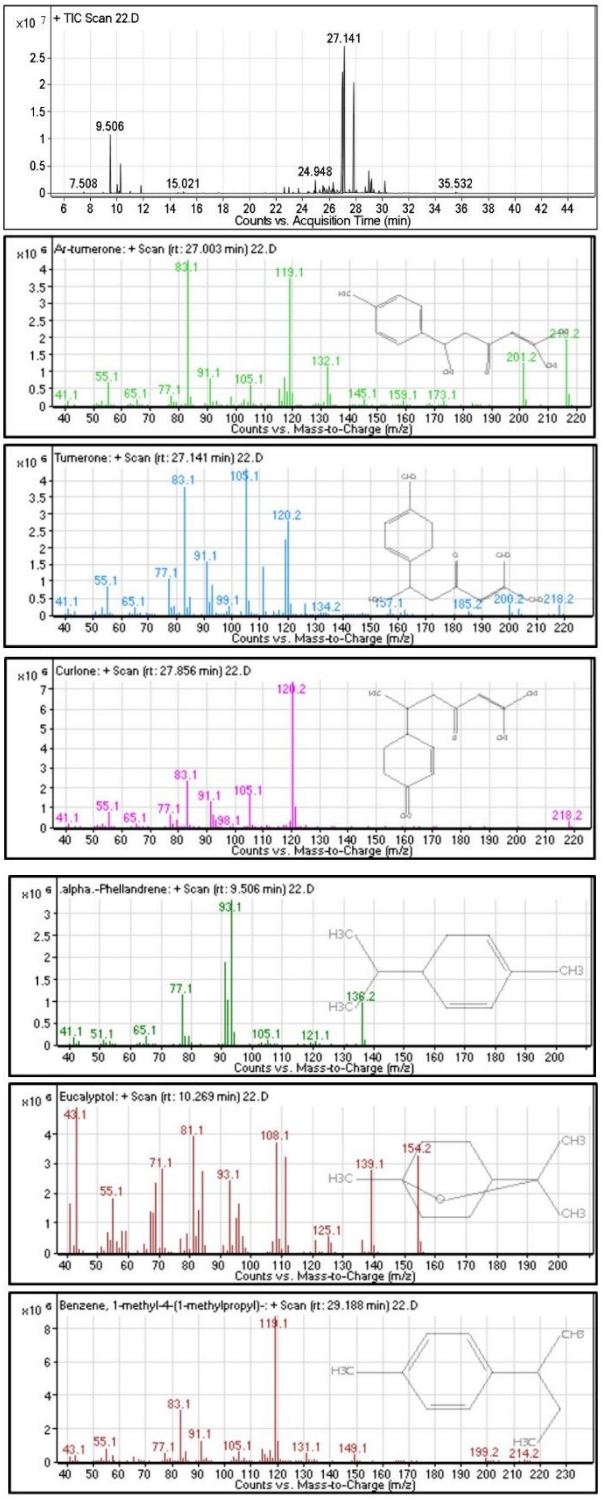

Figure 6. GC/MS chromatogram of Rhizome oil of *Curcuma longa and* Mass spectrum and structure of individual compounds ar-tumerone, tumerone, Curlone, alpha. -Phellandrene, Eucalyptol and Benzene, 1-methyl-4-(1-methylpropyl).

turmeric. While the diversity in these characteristics highlights the genetic variations within turmeric, the intricacies of their relationship with the plant's medicinal attributes remain elusive. It is noteworthy that, despite the apparent influence of genotype-

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Table 1. Identification of volatile bioactive compounds in Erode local genotype by high resolution accurate mass analysis.

No.	Compound name	Retention time (min)	Observed Molecular Mass	Exact Molecular Mass	Ratio (m/z)	Composition (%)
1	Tumerone	27.141	218	218.2	218.2	26.5
2	Ar-tumerone	27.003	216	216.2	216.2	25.4
3	Curlone	27.856	218	218.2	218.2	15.8
4	alpha. -Phellandrene	9.506	136	136.2	136.2	5.1
5	Eucalyptol	10.269	154	154.2	154.2	2.8
6	Tumerone	28.985	234	234.2	234.2	2.6
7	Benzene, 1-methyl-4-(1-methylpropyl)-	29.188	214	214.2	214.2	1.7
8	Tricyclo [2.2.1.0(2,6)] heptane-3-methanol, 2,3-dimethyl-	30.195	216	216.2	216.2	1.5
9	6-(p-Tolyl)-2-methyl-2-heptenol, trans-	25.988	200	200.2	200.2	1.4
10	Benzene, 1,1'-(1,1,2,2-tetramethyl-1,2-ethanediyl) curc-	24.948	200	200.2	200.2	1.4
11	6-(p-Tolyl)-2-methyl-2-heptenol, trans-	25.533	216	216.2	216.2	1.3
12	Lanceol, cis	26.304	202	202.2	202.2	1.3
13	Propanedinitrile, dicyclohexyl-	29.139	234	234.2	234.2	1.1
14	7-(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxatricyclo [5.1.0.0(2,4)] octane	26.605	202	202.2	202.2	0.9
15	Santalol, cis,.alpha.-	25.638	218	218.2	218.2	0.9
16	Lanceol, cis	27.515	204	204.2	204.2	0.8
17	o-Cymene	10.026	134	134.1	134.1	0.8
18	(+)-4-Carene	11.813	136	136.2	136.2	0.8
19	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6 methylene-, [S- (R*, S*)]-	23.681	204	204.2	204.2	0.7
20	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	22.617	202	202.2	202.2	0.6
21	7-Oxabicyclo [4.1.0] heptane, 1-(2,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl-, (E)-	28.717	220	220.2	220.2	0.6
22	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S- (R*, S*)]-	22.958	204	204.2	204.2	0.6
23	cis-sesquisabinene hydrate	25.825	204	204.2	204.2	0.6
24	cis-sesquisabinene hydrate	26.223	204	204.2	204.2	0.5
25	Spiro[bicyclo[3.3.0]octan-6-one-3-cyclopropane]	29.757	218	218.2	218.2	0.5
26	7-Oxabicyclo [4.1.0] heptane, 2,2,6-trimethyl-1-(3-methyl-1,3-butadienyl)-5-methylene-	29.359	218	218.2	218.2	0.5
27	3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (E, E)-	28.051	218	218.2	218.2	0.5
28	4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo [5.1.0.0(2,4)] octane	26.378	218	218.2	218.2	0.4
29	cis-sesquisabinene hydrate	25.281	218	218.2	218.2	0.4
30	Tumerone	24.859	200	200.2	200.2	0.3
31	Caryophyllene oxide	24.42	204	204.2	204.2	0.3
32	D-Limonene	10.164	136	136.1	136.1	0.2
33	gamma. -Terpinene	11	136	136.1	136.1	0.2
34	alpha. -Terpineol	15.021	136	136.2	136.2	0.2
35	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	35.532	281	281	281.1	0.2
36	Longipinocarvone	28.839	218	218.2	218.2	0.2
37	beta.-Bisabolene	23.283	204	204.2	204.2	0.2
38	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	7.508	136	136.2	136.2	0.1
39	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	14.607	154	154.2	154.2	0.1
40	beta.-Myrcene	8.97	136	136.2	136.2	0.1

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environment interaction on curcuminoid content, the exact connection between the observed phenotypes and the medicinal qualities of turmeric, particularly related to curcuminoids, remains to be fully understood. Further research is essential to unravel the complex interplay between genetic factors, environmental conditions, and the therapeutic components of turmeric [26]. The secondary metabolites contribute to colour and flavour, playing significant roles as free radical scavengers, hydrogen donors, reducing agents, and singlet oxygen quenchers. Consequently, to mitigate the potential hazards of oxidative damage, the dietary intake of antioxidant phenolics and flavonoids can be considered the primary line of defense against highly reactive toxicants [27]. Ak and Gulcin [12] in 2008 investigated the antioxidative activity of curcumin utilizing several techniques; DPPH, ABTS, and Fe^{3+}–Fe^{2+} transformation methods curcuminoids (curcumin and its related compounds) are a major chemical constituent in turmeric, having a wide range of pharmacological properties including antioxidant, anti-hypertensive [28].

The earlier studies by Madhusankha et al., [29] did a similar type of work with five turmeric genotypes and observed that visual color differences might range from bright yellow to orange-yellow and proposed that these color differences might be due to the presence of bright yellow curcuminoid pigments. The ratio a*/b* has been used as a color index in apple, tomato, citrus, carambola, and mango fruit [18]. Asghari et al., [30] analysis of curcumin through UFLC revealed an initial increase in curcumin content up to the sixth month, followed by a subsequent decrease during the ninth month. Active vegetative growth was observed in the fourth and eighth months, with a higher quantity of curcumin recorded during the sixth to eighth months. However, no significant difference was observed during the eighth to tenth months. Upon a detailed analysis, our results indicated the highest curcumin content during the ninth month, followed by the sixth and third months. The GC-MS analysis successfully identified and quantified the compounds present in the essential oil, providing a comprehensive profile of the chemical composition of *C. longa* rhizome essential oil. The chemical characterization revealed major 40 compounds in the essential oil of *C. longa*. Previously, Jaiswal and Agrawal [31] reported the identification of various compounds through GC-MS analysis. In their study, GC-MS analysis of *C. longa* rhizomes was conducted using hydro distillation of fresh rhizomes with a clevenger apparatus. The analysis revealed the presence of diverse compounds, including pinene, β-myrcene, α-phellandrene, p-cymene, eucalyptol, γ-terpinolene, α-terpinolene, β-caryophyllene, β-farnesene, γ-curcumene, ar-curcumene, zingiberene, β-bisabolene, α-santalol, β-atlantol, humulene epoxide, zingiberenin, ar-tumerone, curlone, tumerone, artemisia ketone, khusimone, and n-myrcenone. Similarly, Xu et al., [32] identified carvotanacetone, thymol, eugenol, ylangene, β-cubebene, α-cedrene, GC-MS analysis of n-hexane extract of *C. longa* rhizomes and Nuclear Magnetic Resonance (NMR) by Dias Ferreira et al., [33] identified α-pinene and camphor. In 2018, Kumar et al., [34] identified α – thujene, α – terpinene, γ-terpinene using GC-MS analysis of the hydro distillate extract obtained from dried leaves of *C. longa.*

5. Conclusions

This study presents a thorough characterization of turmeric rhizome development, integrating morphological observations biochemical composition of *C. longa* rhizomes, incorporating diverse parameters such as color analysis, pH, protein content, flavonoids, phenolics, tannins, FRAP, and DPPH antioxidant activities. The outcomes contribute valuable insights into the potential health benefits and applications of this botanical resource. The findings contribute valuable insights for optimizing cultivation practices and understanding the bioactive potential of turmeric at different developmental stages.

Abbreviations

ABTS: 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); ACN: Absolute acetonitrile; ANOVA: Analysis of variance; DPPH: 2,2-diphenylpicrylhydrazyl; FRAP: Ferric reducing ability of plasma; GAE: Gallic acid equivalent; GC-MS: Gas chromatography-mass spectrometry; HPLC: High-performance thin-layer chromatography; IC_{50}: Half maximal inhibitory concentration; NMR: Nuclear magnetic resonance; QE: Quercetin equivalent; UFLC: Ultra-fast liquid chromatography.

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Authors' contributions

Experimental conduct, S.G.M.R.; Writing and experimental design, R.K.R.; Writing and data analysis, S.R.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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