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Original Article

GC-MS ANALYSIS OF ESSENTIAL OIL OF SOME HIGH DRUG YIELDING GENOTYPES OF TURMERIC (*CURCUMA LONGA* L.)

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ABSTRACT

Objective: The aim of this investigation was to carry out the qualitative evaluation of selected high drug yielding elite genotypes of turmeric to add to their eliteness.

Methods: 131 turmeric genotypes collected from 10 different agroclimatic zones were analysed for curcumin content. Leaves and rhizomes of these plants were collected for extraction of essential oil. Curcumin percentage of the sample was estimated according to the ASTA method. Essential oil was extracted by hydro-distillation of fresh leaves and rhizomes following the method of Guenther (1972). Initial screening of elite genotypes was done on the basis of curcumin content (≥5%), rhizome oil content (≥1.5%) and leaf oil content (≥0.5%). Selected elite genotypes were subjected to qualitative evaluation of essential oil through GC-MS analysis.

Results: The five high rhizome oil yielding genotypes, TR1, TR2, TR3 and TR5 containing high rhizome oil yield of 2.1%, 1.7%, 1.6% and 1.5% respectively were considered to be elite clones containing tumerone as the major constituent of rhizome essential oil along with all desirable constituents. On the basis of leaf oil yield, genotypes TL1 and TL2 with 1.9% and 1.1% leaf oil were proved as elite clones with α–phellandrene as the major constituent along with other desirable constituents. GC-MS analysis of 3 selected high curcumin yielding genotypes TC1, TC2 and TC3 with curcumin content 7.3, 7.2 and 7.0% respectively revealed TC1 and TC2 as elite genotypes containing high quality rhizome and leaf oil.

Conclusion: The present investigation reveals that eight genotypes of turmeric selected with high drug yield and high quality essential oil would have enough significance for boosting the production and export of value added products in the national and international market.

Keywords: *Curcuma longa,* Genotypes, Curcumin, Essential oil.

INTRODUCTION

Turmeric (*Curcuma longa* L.) is a unique plant combining properties of a spice, colourant, cosmetic and a drug useful in a number of diseases. Turmeric is cultivated most extensively in India, Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia, Philippines, Australia, Africa, Peru and West Indies. Recently, turmeric has received considerable attention for its therapeutic importance. Turmeric contains various biologically active substances which demonstrate antioxidant, anti-tumour, germicidal, aromatic, carminative, anti helmentic, cholesterol lowering and neuroprotective activities [1-10]. The drug yielding potential is due to the presence of curcumin and essential oil present in it. Curcumin present in the rhizome is used for rheumatoid arthritis [11], multiple sclerosis [12] and Alzheimer's disease [13, 14]. It also protects from liver injury [15], increases bile secretion [16], protects from cataract formation [17] whereas essential oil present in rhizome and leaves have varied properties like anti microbial, anti-inflammatory, anti-wounds, anti-dermatosis, insect repellant and used in various digestion ailments [18, 19]. Cost of curcumin in an international market is approximately 13.5$ per 100g whereas cost of 10 ml of rhizome oil and leaf oil is 3$ and 1$ respectively. Therefore elite genotype of turmeric with high curcumin yield and high essential oil yield are of high demand.

Elite genotypes identified so far in turmeric through time to time survey [20-22] are mostly on the basis of oil and curcumin yield. Reports on qualitative evaluation of essential oil of turmeric showing details of chemical constituent is very limited [23-25]. Drugs containing desirable chemical constituents have high export potential. Thus it was essential to carry out the qualitative evaluation of selected high drug yielding elite genotypes to add to their eliteness. The present work of GC-MS analysis of chemical constituents of essential oil of high drug yielding genotypes of turmeric is of enough commercial significance.

MATERIALS AND METHODS

Plant material

The present investigation deals with 131 turmeric genotypes collected from 10 different agroclimatic zones out of total 14 agroclimatic zones available in India. They were maintained in field gene banks of Centre of Biotechnology of Siksha 'O' Anusandhan University. Rhizomes of all the accessions and cultivars of turmeric collected from different agroclimatic regions were analysed for curcumin content. These plants were grown in medicinal plant garden for collection of leaves and rhizomes for extraction of essential oil.

Extraction and quantification of curcumin

The rhizomes were cut into small pieces and dried in the air. The air dried rhizomes were powdered in a mortar with a pestle, 0.1 g of powdered rhizome was taken in a flat bottom flask and 75 ml of acetone was added and refluxed for 4 h. The refluxed residue was cooled and taken on a filter paper, washed with 100 ml of acetone. A 10 ml of the filtrate was taken and diluted to 250 ml with acetone. The absorbance of the diluted sample and that of the standard curcumin (95% HPLC Purified, purchased from Charak International) solution was measured at 420 nm by spectrophotometer and curcumin percentage of the sample was estimated according to the ASTA method (Method no. 1.09, 1997) [26]:

$$\frac{\text{Absorbance of the sample} \times \text{dilution factor}}{\text{Factor from standard} \times \text{weight of the sample}}$$

Extraction and quantification of essential oil

Essential oil was extracted by hydro-distillation of fresh leaves and rhizomes of field grown turmeric plants of different agroclimatic regions, in a Clevenger's apparatus following the method of Guenther [27]. The fresh leaves and rhizomes of turmeric were

washed to remove soil, peeled and sliced. Sliced leaves and rhizomes of fresh turmeric (100 g) were mixed with distilled water. A flask containing the sliced leaves and rhizomes was heated for 3–4 h for leaf oil and 6–10 h for rhizome oil and the condensed vapour was separated throughout an auto-oil/water separator. The oil present at the upper most layers was collected in the eppendorf tube. Each essential oil extraction was run in triplicate.

Oil yield percentage in leaves and rhizomes was calculated by following method. Yield percentage was recorded as fresh weight basis.

$$\text{Leaf oil \% yield (v/w)(fresh weight)} = \frac{\text{volume of essential oils (ml)}}{\text{weight of raw leaves taken}} \times 100$$

$$\text{Rhizome oil \% yield (v/w)(fresh weight)} = \frac{\text{volume of essential oils (ml)}}{\text{weight of raw rhizomes taken}} \times 100$$

GC-MS analysis of essential oil

The GC MS analysis was performed by Perkin Elmer GC-MS (model: Clarus 580, USA) equipped with a SQ8S MS detector fused with Elite-5 capillary column (30m×0.25 mm i.d., film thickness 0.25 µm). For detection purpose electron ionisation principle with 70eV ionization energy was used. Helium gas was used as a carrier gas, at a constant flow rate 1 ml/min. The oven temperature was programmed as 60 °C to 120 °C at 3 °C per min., then raised to 170 °C at 5 °C/min., finally raised to 215 °C at 30 °C/min. The final run time was 45 min. having mass scan range 50-600 amu with 4 min. solvent delay. 0.5 µl of sample was injected for performing analysis. The identification of phytoconstituents of different samples was carried out by comparing mass spectra of compounds present in NIST library (NIST-2011, Mainlab GC-MS Systems).

Selection of high drug yielding genotypes for qualitative evaluation

Elite turmeric genotypes were screened on the basis of desired drug yielding traits (curcumin content ≥ 5%, leaf oil content ≥ 0.5% and rhizome oil content ≥ 1.5%) for qualitative evaluation through GC-MS analysis.

RESULTS AND DISCUSSION

Selection of turmeric genotypes with high curcumin content

The rhizomes collected from all 131 turmeric germplasms from different agroclimatic regions were analysed for curcumin content. The analysis revealed that the content of curcumin in the dried rhizomes varied from 0.7%-7.3% among all turmeric genotypes. Rama Rao and Rao [29] also reported that phytoconstituent of turmeric varied from place to place due to influence of environment and agro-climatic conditions. Out of 131 germ plasms analysed, 3 genotypes having curcumin content>5% were selected. The selected genotypes were TC1, TC2 and TC3 from North Eastern Ghat region having curcumin content of 7.3%, 7.2% and 7.0% respectively (table 1).

Table 1: Selected elite genotypes of turmeric with high curcumin, high leaf oil and high rhizome oil content

Selected genotype	Agroclimatic zone of collection	Elite drug yield trait	Percentage yield (%)
TR1	North Eastern Ghat	High rhizome oil content	2.1
TR2	North Eastern Ghat	High rhizome oil content	1.7
TR3	Eastern Ghat Highland	High rhizome oil content	1.6
TR4	North Eastern Ghat	High rhizome oil content	1.5
TR5	North Eastern Ghat	High rhizome oil content	1.5
TC1	North Eastern Ghat	High Curcumin content	7.3
TC2	North Eastern Ghat	High Curcumin content	7.2
TC3	North Eastern Ghat	High Curcumin content	7.0
TL1	South Eastern Ghat	High leaf oil content	1.9
TL2	Eastern Ghat Highland	High leaf oil content	1.1

Evaluation and selection of high essential oil yielding turmeric

The oil was obtained by hydrodistillation of the leaves and rhizomes of turmeric in a Clevenger apparatus. From the analysis, it was observed that the content of essential oil in the rhizomes varied from 0.6% to 2.1% among 131 samples analysed. The content of essential oil in the leaf varied from 0.2% to 1.9%. Out of 131 germ plasms analysed, 5 genotypes such as TR1, TR2, TR3, TR4, TR5 were selected as high rhizome oil containing turmeric with 2.1%, 1.7%, 1.6%, 1.5% and 1.5% oil contents respectively (table 1). Similarly, two genotypes such as TL1 from South Eastern Ghat zone and TL2 from Eastern Ghat highland zone were selected as high leaf oil containing turmeric with 1.9% and 1.1% leaf oil content respectively (table 1). Besides few elite cultivars and accessions reported by us [21], most of turmeric genotypes reported so far have shown rhizome oil content up to 1% and leaf oil content upto 0.5% [23, 29]

GC-MS based qualitative evaluation of high rhizome oil yielding turmeric

Essential oil of rhizomes from 5 promising elite genotypes of turmeric with high rhizome oil yield was selected for GC-MS based qualitative analysis (table 2). From GC-MS analysis of essential oil, tumerone was identified as the major compound occupying maximum peak area in all genotypes of turmeric from different agro-climatic zones. Percentage of tumerone varied from 35.24% to 44.22% among all five elite genotypes. Tumerone was 35.24% (lowest) in TR4 and 44.22% (highest) in TR5. A similar result has also been found in *Curcuma aromatica* rhizome oil of different agro-climatic regions of Bangladesh by Al-reza *et al.* [30]. Varying percentage (16.7-25.7%) of turmerone as the major compound, in rhizome oil of different cultivars of *C. longa* has been reported by Sharma and Singh [31]. Our results match greatly with the study of Nigam and Ahmad [32]. They analyzed the rhizome oil of *C. longa* from different agroclimatic regions of Lucknow. Other desirable chemical constituents like α-phellandrene, eucalyptol, terpinolene, α-fernasene etc. were also found in the rhizome essential oil of 5 selected genotypes except TR4 lacking presence of 3 compounds (table 2).

GC-MS based qualitative evaluation of high leaf oil yielding turmeric

Essential oils of leaves of two high leaf oil yielding turmeric genotypes (TL1 and TL2) were selected for GC-MS analysis. From GC-MS analysis of essential oil, α-phellandrene was identified as the major compound in both of the genotypes. Percentage of α–phellandrene which comprised maximum peak area in leaf oil, was 30.82% in acc TL1 to 39.85% in acc TL2 (table 3). α-Phellandrene was reported as the major chemical constituent in turmeric leaf oil of different origin [33-36]. Other desirable chemical constituents like eucalyptol, terpinolene, terpinene, β-pinene, zingiberene were also found in the leaf essential oil of both genotypes.

GC-MS based qualitative evaluation of essential oil of high curcumin yielding turmeric

Essential oil of leaves and rhizomes from 3 high curcumin containing elite genotypes of turmeric was selected for GC-MS analysis. From GC-MS analysis of leaf oil of 3 elite genotypes, it was observed that α-phellandrene was the major compound. Percentage of α-phellandrene was 32.49%, 38.06% and 39.06% in TC1, TC2 and TC3 respectively (table 4). Leaf oil of TC1 and TC2 contained all desired constituents, some of which were missing in TC3 (table 4). GC-MS

analysis of essential oil of rhizome showed the presence of tumerone as a major compound in all the 3 high curcumin yielding genotypes. Percentage of tumerone was 48.71, 55.13 and 32.28 % in TC1, TC2 and TC3 respectively. Rhizome oil of TC1 and TC2 comprised all desirable constituents but in TC3 some constituents were not found (table 5).

Table 2: Chemical constituents of essential oil of high rhizome oil yielding turmeric

Compound	RT	Area %				
		TR1(Mean±SD)	TR2(Mean±SD)	TR3(Mean±SD)	TR4(Mean±SD)	TR5(Mean±SD)
Bergamotene	7.252	-	0.23±0.05	0.30±0.05	1.13±0.09	0.43±0.03
α-Phellandrene	7.985	1.95±0.1	6.25±0.2	7.08±0.1	2.63±0.08	5.47±0.1
P-Cymene	8.591	0.54±0.07	0.36±0.07	0.36±0.04	2.35±0.09	1.29±0.09
Eucalyptol	8.884	0.64±0.09	5.41±0.1	3.32±0.1	2.60±0.07	3.38±0.1
Terpinolene	10.754	0.77±0.08	0.70±0.08	3.09±0.1	2.34±0.08	0.38±0.05
β Thujene	13.999	2.81±0.1	1.24±0.08	0.72±0.07	2.85±0.09	0.48±0.04
Terpineol	15.246	2.71±0.1	2.05±0.07	3.02±0.05	3.48±0.07	0.45±0.07
Camphene	15.283	2.02±0.09	0.30±0.03	0.39±0.05	-	0.43±0.06
O-Cymene	15.631	2.19±0.09	0.33±0.04	0.35±0.04	-	0.30±0.01
Cis-α-Bisabolene	25.551	0.38±0.03	0.36±0.05	0.31±0.05	-	0.34±0.08
α-Curcumene	26.412	1.29±0.08	1.45±0.08	0.73±0.06	0.77±0.08	1.12±0.1
Y-Himachelene	26.853	2.66±0.1	5.48±0.2	1.64±0.1	1.13±0.1	2.96±0.2
α-Fernasene	27.164	1.73±0.09	0.68±0.08	2.31±0.1	1.63±0.1	0.51±0.08
Tumerone	31.491	41.38±0.3	43.42±0.4	41.63±0.2	35.24±0.3	44.22±0.4

RT-retention time, M-mean and SD-standard deviation

Table 3: Chemical constituents of essential oil of high leaf oil yielding turmeric

Constituents	RT	TL1(Mean±SD)	TL2(Mean±SD)
α-Phellandrene	5.451	30.82±0.2	39.85±0.3
Eucalyptol	6.172	7.52±0.1	7.66±0.1
Terpinolene	7.964	26.59±0.3	25.74±0.2
α-Terpinene	7.068	1.08±0.09	1.63±0.09
β-Pinene	5.089	0.77±0.07	1.31±0.08
β-Myrcene	5.203	0.87±0.08	0.91±0.07
o-Cymene	23.843	2.18±0.1	1.85±0.1
2-Carene	8.417	0.96±0.09	0.89±0.08
Zingiberene	23.843	2.15±0.08	2.09±0.09
β-Sesquiphellandrene	24.900	1.0±0.1	1.03±0.1
Tumerone	30.342	1.39±0.1	1.41±0.1

RT-retention time, M-mean and SD-standard deviation

Table 4: Chemical constituents of leaf essential oil of high curcumin yielding turmeric

Constituents	RT	TC1(Mean±SD)	TC2(Mean±SD)	TC3(Mean±SD)
α-Phellandrene	5.588	32.49±0.2	38.06±0.3	39.06±0.2
Eucalyptol	6.277	8.67±0.1	12.35±0.1	-
Terpinolene	8.670	18.81±0.3	19.43±0.2	17.71±0.1
β-Pinene	4.759	1.43±0.09	1.03±0.09	-
β-Myrcene	5.224	1.61±0.07	1.43±0.08	1.74±0.06
p-Menthatriene	6.108	3.88±0.09	2.37±0.1	-
3-Carene	6.112	3.29±0.3	3.31±0.2	3.41±0.2
Zingiberene	23.851	2.1±0.07	0.33±0.09	-
Tumerone	30.351	0.58±0.04	0.53±0.07	4.79±0.09
o-Cymene	6.007	2.55±0.1	2.37±0.1	21.03±0.3
4-Carene	8.066	24.34±0.3	25.17±0.2	-

RT-retention time, M-mean and SD-standard deviation

Table 5: Chemical constituents of rhizome essential oil of high curcumin yielding turmeric

Constituents	RT	TC1(Mean±SD)	TC2(Mean±SD)	TC3(Mean±SD)
Tumerone	30.562	48.71±0.2	55.13±0.2	39.28±0.2
Eucalyptol	6.273	1.9±0.1	5.71±0.1	-
α-Curcumene	23.353	1.76±0.1	2.78±0.1	2.02±0.1
α-Zingiberene	23.860	1.73±0.1	2.49±0.1	2.31±0.1
Curlone	31.670	15.71±0.2	14.12±0.2	18.05±0.2
Bisabolene	24.359	0.84±0.04	0.70±0.03	-
Sesquiphellandrene	24.959	5.12±0.1	4.09±0.1	2.92±0.1
α-Phellandrene	5.432	0.60±0.03	0.78±0.02	-
o-Cymene	6.176	1.78±0.1	1.64±0.09	-

RT-retention time, M-mean and SD-standard deviation

Selection of elite genotypes of turmeric with high yield and quality

GC-MS analysis was carried out with selected 10 high drug yielding turmeric genotypes out of 131 germ plasms collected from different agroclimatic regions for quality evaluation of their essential oil. Of the five high rhizome oil yielding genotypes, TR1, TR2, TR3 and TR5 containing high rhizome oil yield 2.1%, 1.7%, 1.6% and 1.5% respectively were considered to be elite clone containing tumerone as the major constituent of rhizome essential oil along with all desirable constituents (table 2, fig.1). On the basis of leaf oil yield, genotypes TL1 and TL2 with 1.9% and 1.1% leaf oil were proved to be the best clones with α–phellandrene, the major constituent along with other desirable constituents (table 3, fig. 2). GC-MS analysis of 3 selected high curcumin yielding genotypes TC1, TC2 and TC3 with curcumin content 7.3, 7.2 and 7.0% respectively revealed TC1 and TC2 as elite clones.

Both the rhizome and leaf oil yield of these two genotypes possessed all the desirable constituents (table 4&5, fig.3 & 4). Important chemical constituents present in leaf and rhizome oil have varied properties like anti microbial, anti inflammatory, anti wounds, anti dermatosis, insect repellant etc and are used in various digestion ailments [18, 19, 37] and thus adding to the eliteness of 8 selected high drug yielding elite genotypes of turmeric with high quality essential oil.

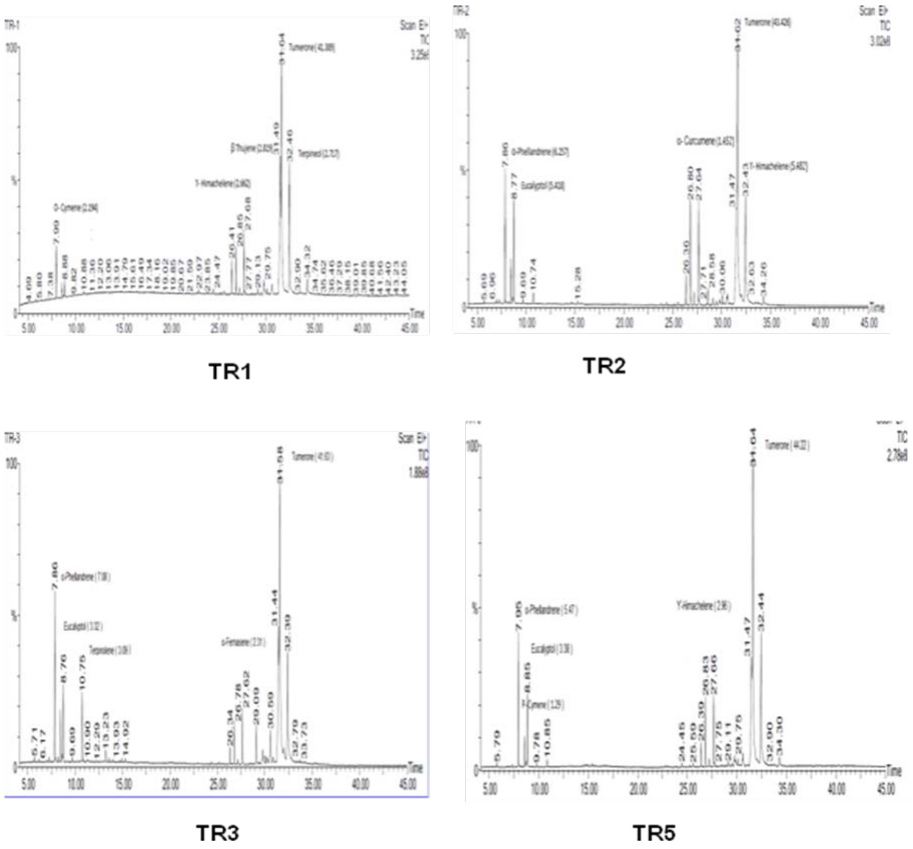


Fig. 1: GC-MS spectrum of high rhizome oil yielding turmeric (TR1, TR2, TR3, TR5)

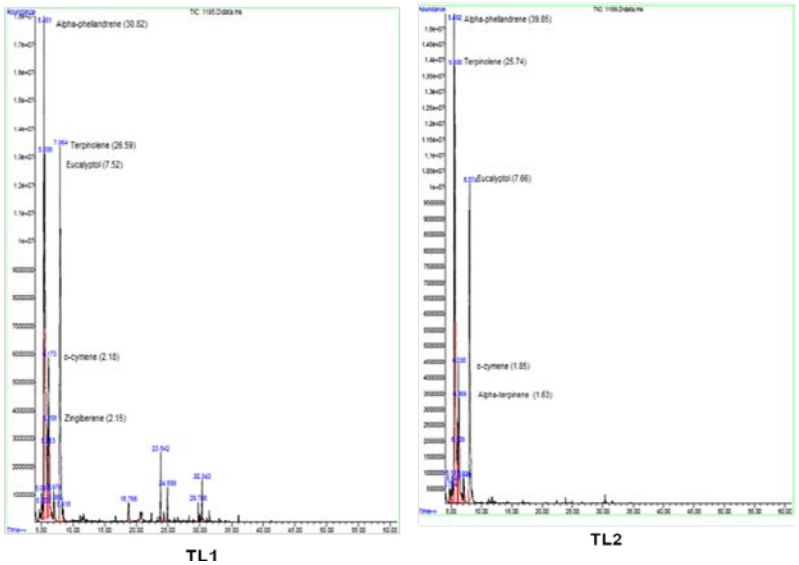
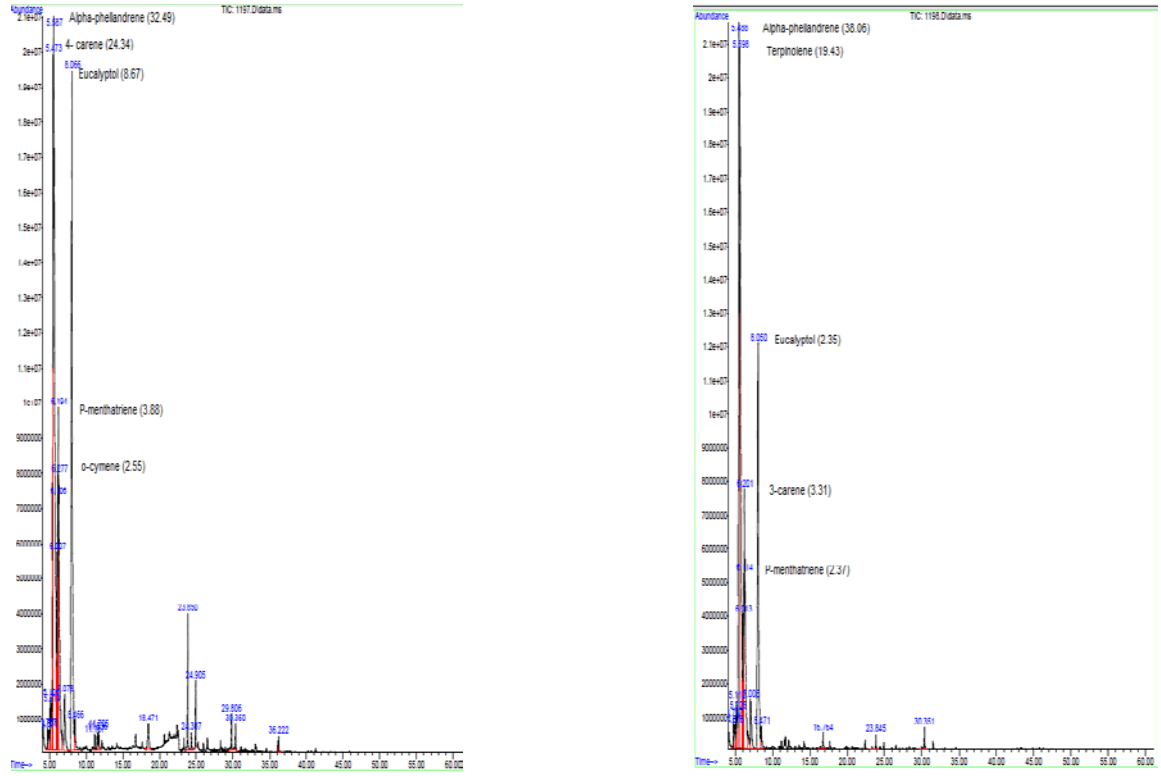
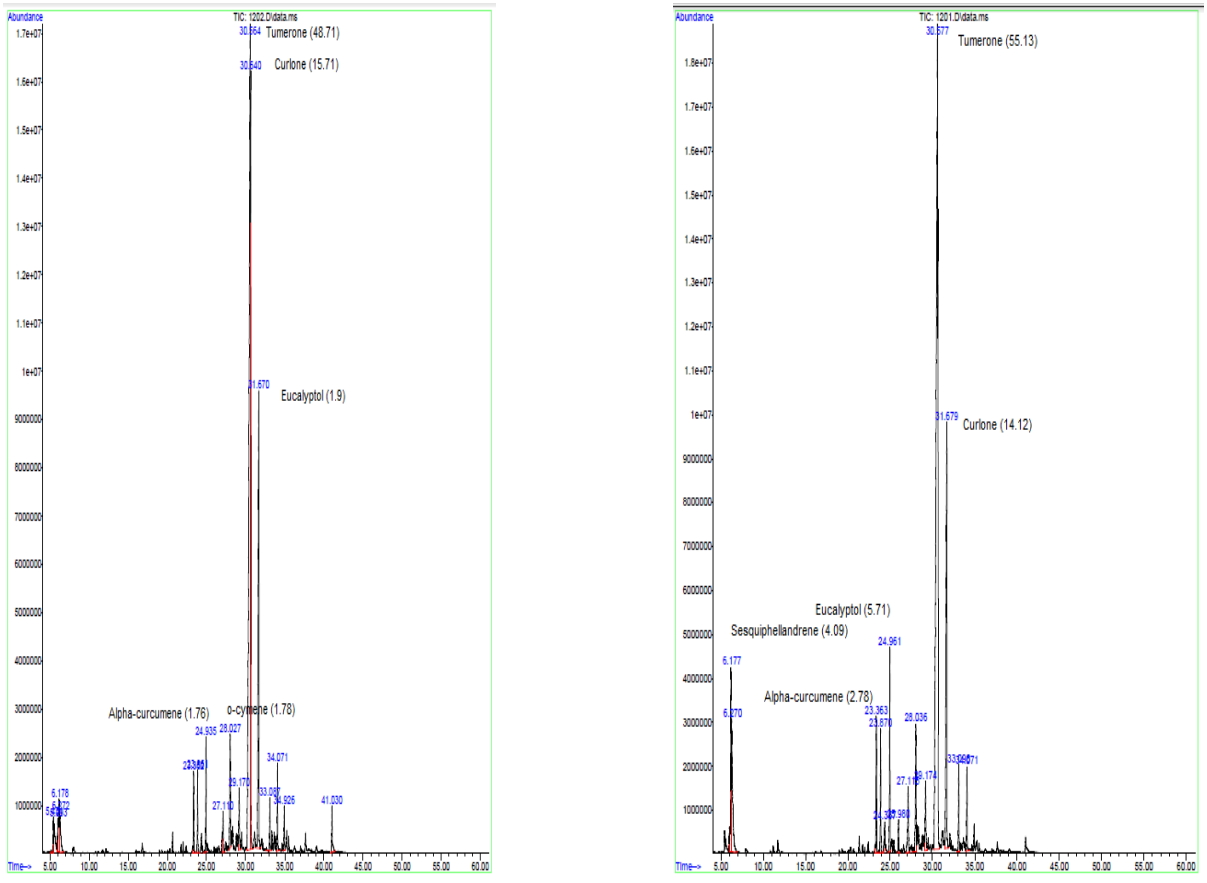


Fig. 2: GC-MS spectrum of high leaf oil yielding turmeric (TL1, TL2)



TC1 **TC2**

Fig. 3: GC-MS spectrum of leaf essential oil of high curcumin yielding turmeric (TC1) & (TC2)



TC1 **TC2**

Fig. 4: GC-MS spectrum of rhizome essential oil of high curcumin yielding turmeric (TC1) & (TC2)

CONCLUSION

These eight genotypes of turmeric selected with high drug yield and high quality essential oil would have enough significance for boosting the production and export of value added products in the national and international market and they can be released as new cultivars after subsequent analysis.

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CONFLICT OF INTERESTS

Declared None

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