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Biochemical studies in different varieties of turmeric (*Curcuma longa* L.)

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Abstract

An experiment on extraction of essential oil and curcumin content was carried out at chemistry lab of D.A.V. College, Kanpur. Turmeric samples were collected from A.N.D. University of Agriculture and Technology Kumarganj, Ayodhya (U.P.) and some biochemical parameters viz. Moisture content, Total mineral content, Carbohydrate content, Crude Fiber content, Curcumin content, Oleoresin content and Essential oil content are studied in different varieties (N.D.H.-1, NDH-2, NDH-3, NDH-98 and Prabha) of turmeric (*Curcuma longa*). Moisture content ranged from 74.98% to 85.03% and maximum moisture was found in NDH-1 (85.03%). Total mineral content ranged from 7.01 ± 0.34 to 12 ± 0.34 % and maximum total mineral content was found in NDH-1 (12 ± 0.34 %). Crude fiber content varied with variety to variety. It ranged from 4.64 to 5.65%. Maximum Crude Fiber (%) in fresh Rhizome was found in NDH-2 (5.65%) followed by NDH-98 (5.6%), NDH-3 (4.75%), NDH-1 (4.65%) and the lowest crude fiber content was found in Prabha (4.64%). Carbohydrate content ranged from 61.50 to 70.40% and maximum Carbohydrate was found in NDH-2 (70.40%). Curcumin content varied from (2.22%) to (8.44%), maximum curcumin content was recorded in NDH-2 (8.442%). Oleoresin content ranges from 5.7% to 21.9% and maximum content was found in NDH-2 (21.9%). Essential oil content ranged from 1.63 to 3.91%. And maximum essential oil content was found in Prabha (3.1%). The results obtained from the analysis of fresh turmeric rhizome indicated that maximum moisture was found in NDH-1 (85.03%), maximum total mineral content was found in NDH-1 (12 ± 0.34 %), Maximum Crude Fiber (%) in was found in NDH-2 (5.65%), maximum Carbohydrate in NDH-2 (70.40%), maximum curcumin content in NDH-2 (8.442%), maximum oleoresin content was found in NDH-2 (21.9%) while highest essential oil content in Prabha (3.91%) among all five varieties.

Keywords: Turmeric, moisture, mineral, carbohydrate, crude fiber, oleoresin, curcumin, essential oil, variety

Introduction

Turmeric (*Curcuma longa* L.) belongs to the Zingiberaceae family grown in warm rainy regions of the world such as India, China, Indonesia, Jamaica and Peru. India is the world's largest producer and exporter of turmeric (90% of the world's total production). The country produced about 946mt of turmeric from approximately 257mha area and 3683kg/ha productivity of crops during 2019-2020 (Agricultural Statistics at a Glance Directorate of Economics and Statistics, New Delhi, September, 2020). The main turmeric growing states are Andhra Pradesh, Maharashtra, Orissa, Tamil Nadu, Karnataka and Kerala. Andhra Pradesh occupied the largest area coverage 36% with 47% production share in India. That means Andhra Pradesh topped in both area and production of turmeric. The second largest area is covered by Tamil Nadu at 16%, with a production share of 21%. In Ayurveda medicine, turmeric is primarily used as a treatment for inflammatory conditions and in traditional Chinese medicine, it is used as stimulant, aspirant, carminative, cardial, astringent, detergent, diuretic and martinet (Remadevi *et al.*, 2007). Turmeric has also been used for centuries as traditional remedies such as stimulant, stomachic, carminative, diuretic, anti-diarrhoea, anti-emetic, anti-inflammatory, antipyretic, anti-microbial and antioxidant agent (Jayaprakash *et al.*, 2005) [6]. The use of turmeric as a spice and as a household remedy has been known to be safe for centuries. To date, no studies in either animals or humans have discovered any toxic effects associated with the use of turmeric (Lao *et al.*, 2006) [11]. In a clinical study on the safety and tolerance of turmeric oil use, the oil was administered orally to healthy volunteers for 3 months. No side effects of turmeric oil intake were observed in 3 months on body weight, blood pressure, and hematological, renal, or hepatic toxicity (Joshi *et al.*, 2003). The main component of turmeric is named curcuminoids, which include mainly curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin (Chainani-Wu, 2003) [2].

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Curcumin is a secondary metabolite and the most important fraction of turmeric which is responsible for the biological activities. The melting point of curcumin, C₂₁ H₂₀O₆, is 184 °C. It is soluble in ethanol and acetone, but insoluble in water (Joe *et al.*, 2004) [7]. Curcumin was first isolated in 1815 (Vogel & Pelletier, 1815) [24] and its structure was determined by Roughley and Whiting in 1973 [23].

The total of curcuminoids which is about 7-10%, turmeric also contains 2-4 per cent essential oil and 2-3 per cent of fixed oil and various volatile oils, including turmerone, atlantone, and zingiberone. Other constituents include sugars, proteins and resins. The value of the turmeric products is based on their curcuminoids content and estimated based on its absorbance at 420 nm (Merina Benny Antony., 2003) [16].

Curcuminoids are polyphenols having a pronounced yellow colour. Curcumin is unstable at basic pH and degrades within 30 minutes to trans-6-(4-hydroxy-3-methoxyphenyl) - 2, 4 dioxo-5-hexanal, ferulic acid, ferulomethane and vanillin. Under acidic conditions, the degradation of curcumin is much slower, with less than 20% of total curcumin decomposed at 1 hrs (Lin *et al.*, 2000) [14]. Curcuminoids are soluble in dimethyl sulfoxide (DMSO), acetone and ethanol. They are readily decomposed when exposed to bright light, high temperature or oxidative conditions (Schieffer, 2002) [26]. The content of curcuminoids may vary in turmeric rhizome grown in different agro-climatic zones (Revathy *et al.*, 2011) [22]. The curcuminoids, which consist mostly of curcumin 1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione and also demethoxy- curcumin and bisdemethoxycurcumin. The essential oil extracted from turmeric oleoresin has been reported to have antibacterial, antioxidant and antifungal activities (Vijayastelter *et al.*, 2011) [29]. Due to its ability to preserve food through its antioxidant activity, to give colour and taste to the food, its health promoting effects are less well recognized or appreciated and for being anti-microbial, it is used extensively for cosmetic applications. Turmeric is the only spice which finds application in all the three segments of life *i.e.* food, cosmetics and health. Lokhande *et al.* (2013) [15] observed no significant difference in the moisture content of different Indian cultivars (Salem, Krishna and Tekurpetha). Lokhande *et al.* (2013) [15] who reported that carbohydrate content of all cultivars was in the range of 67.9 to 69.9 per cent. Lokhande *et al.* (2013) [15] who reported that crude fibre content of all cultivars was in the range of 7.19 to 8.06 per cent and maximum value was in Tekurpetha followed by Salem and Krishna. Sasikumar *et al.* 1996 [25] who observed that IISR varieties Prabha and Pratibha had 15.0 to 16.2% oleoresin content respectively. Lokhande *et al.* 2013 [15] reported significant difference in all the recent three cultivars. Sasikumar *et al.* (1996) [25] observed that IISR Prabha and Pratibha had 6.25 and 6.21 per cent curcumin content, respectively. Whereas, highest range of curcumin percentage was 3.584 to 7.730% in Pratibha followed by Salem 2.169 to 5.932%, Rajapuri 2.812 to 4.366%, Krishna 1.599 to 3.520% respectively, as observed by Kamble *et al.*, 2011. Factors such as the extraction method, solvent used for extraction, extraction time and temperature, the solvent ratio and extraction pressure were among the significant factors that were shown to be able to influence the efficiency of curcumin extraction (Wakte *et al.*, 2011) [30]. Curcumin content was reported to vary from one species to another. Several studies have shown that soil factors, including nutrients and level acidity as well as the genus diversity, may affect the content

of curcumin (Nahak and Sahu, 2011) [17].

Methods and Material

Moisture content in turmeric rhizome

The moisture content in turmeric rhizome was estimated by Rangana (1986) 100g of fresh rhizome were taken in petridish and placed in an oven at a temperature of 70°C for 16 to 18 hours. Weight of dried rhizome samples was taken and moisture percent was calculated by using the following formula:

$$\text{Moisture (\%)} = \frac{\text{weight of sample} - \text{weight of dry sample}}{\text{weight of sample}} \times 100$$

Estimation of Total Mineral Content (%)

The total mineral content was estimated by the methods as described by Hart and Fisher (1971). 0.2g oven dried sample was dried at 70 °C temperature and transferred into ashless filter paper. The ignition of sample was carried out on non-luminous flame in a preweighed treated crucible. The crucible was finally placed into muffle furnace, which was maintained at 525-550 °C for about 5-6 hours to destroy the organic matter of the sample. After expiry of the period the crucible was transferred into a desiccator for cooling to avoid absorption of moisture by ash. The cooled ash along with silica crucible was weighed. The result was calculated and reported on moisture free basis and expressed in percent.

$$\text{Total mineral content (\%)} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

Where

Weight of ash= W₂ - W₁

W₁= Weight of crucible + sample

W₂= Weight of crucible after removing from the oven

Estimation of Carbohydrate:

Sample Preparation

1. Take 100mg of the sample into a boiling tube.
2. Hydrolyse by keeping it in boiling water bath for 3 hours with 5mL of 2.5 N-HCl and cool to room temperature.
3. Neutralise it with solid sodium carbonate until the effervescence ceases.
4. Make up the volume to 10mL and centrifuge.
5. Collect the supernatant and use for further analysis.

Assay Procedure

1. Prepare five to eight dilutions of a Glucose standard with a range of 5 to 30 µg/ml.
2. Add 50 µl standard Glucose Solution or 50 µl unknown sample to an appropriately labeled test tube.
3. Set two blank tubes. For the standard curve, add 50 µl PBS (phosphate-buffered solution) instead of the standard solution. For the unknown samples, add 50 µl preparation buffer instead.
4. Add 1ml of Anthrone reagent to each tube and mix well.

Estimation of Curcumin Content in Turmeric Rhizome Preparation of standard graph of curcumin

Standard curve was obtained using the standard solution in the range of 1µg/ml to 4µg/ml (about 40% -160% of the standard concentration of 0.5 µg/ml).

Absorbance of these solutions were taken at 425 nm using UV-visible spectrophotometer.

Preparation of crude turmeric rhizome powder

Rhizomes were dried and grounded to fine powder.

Preparation of Sample solution

- 10mg crude turmeric rhizome fine powder was dissolved in 5ml of 95% alcohol.
- Mixture was properly shaken in rocker for 1 hour and volume made upto 10ml with 95% alcohol.
- Mixture was filtered by Whatman filter paper and diluted 20X before reading.

Absorbance was measured at 425nm in visible spectrophotometer.

Estimation of Crude Fibre Content (%)

Sample Preparation

The plant materials were powdered with a mechanical grinder to form a coarse powder. The powder was passed through sieve no 40 and was stored in an air tight container until further use. The powder was used for the extraction process.

Procedure

- 2gm of turmeric rhizome powder was defatted and dried. After that sample was boiled with 200 ml of sulphuric acid for 30 min.
- Filtered through filter paper and then washed with boiling water.

- After that sample was digested with 200ml of sodium hydroxide solution for 30 min and filtered through filter paper again and washed with mixture contained 25ml of boiling H₂SO₄, 150ml water and 25ml alcohol.
- Residue was removed and transferred to dish and dried for 2h at 130 ±2°C. Dish was cooled down in a desiccator and weighed and ignited for 30min at 600 ±15°C then cooled in a desiccator and reweighed

Calculation

% Crude fibre = $(W_2 - W_1) - (W_3 - W_1) / \text{Weight of the sample} \times 100$

Estimation of Oleoresin content (%) in Rhizome Sample Preparation

Plant Tissue (50-100gm) was cut down to small pieces (5 mm³) and dried in oven at 37°C for 2-3 days until dry. After drying, the tissue were grounded into powder. Powder was further dried in oven at 37°C until completely dry and stored in cool place.

Procedure

- 10 gm dried plant powder was packed in a paper thimble.
- Soxhlet Apparatus was setup and paper thimble was placed in it.
- 250-300 ml Ethyl acetate was added to the system and allowed to extract for 7-8 cycles at 80°C water bath.
- Extract was dried in oven at 37°C and weighed for further calculations.

$$\text{Oleoresin content (\%)} = \frac{\text{Weight of beaker with oleoresin} - \text{weight of beaker}}{\text{weight of sample}} \times 100$$

Essential oil

The rhizome essential oil content was estimated by hydrodistillation method as described in A.O.A.C. (1970). 10g of powder rhizome were transferred into extractor by using pre-weighted thimble. The thimble was placed into extractor of soxhlet apparatus. The receiving flask of the soxhlet apparatus containing ethanol (40-60°C) was heated on a water bath. Thus, the glass assembly with sample was refluxed on water bath containing 8 to 10 hours for extraction of oil. Finally, the solvent in the receiving flask was distilled off leaving behind pure oil in the flask. The flask was weighed with oil and the observation recorded was calculated as given below:

$$\text{Oil content (\%)} = \frac{W_2 - W_1}{\text{weight of sample}} \times 100$$

$$\text{Curcumin content (g/100g)} = \frac{0.002 \times A_{425} \times \text{volume made up} \times \text{Dilution factor} \times 100}{0.42 \times \text{weight of sample (g)} \times 1000}$$

0.42 absorbance at 425 = 0.0025g curcumin / litre

Result and Discussion

Moisture content in turmeric rhizome

Data in respect of moisture content shown in Table-1 and depicted in fig-1. It was revealed from the data that moisture content varied with variety to variety.

W₁ = Weight of empty flask

W₂ = Weight of flask+ oil

Curcumin content: (Thimmaiah, 1999) [28]

Rhizomes were dried and grounded to fine powder. 10mg crude turmeric rhizome fine powder was dissolved in 5ml of 95% alcohol. Mixture was properly shaken in rocker for 1 hour and volume made upto 10ml with 95% alcohol. Mixture was filtered by Whatman filter paper and diluted 20X before reading. Absorbance was measured at 425nm in visible spectrophotometer. Standard curve was obtained using the standard solution in the range of 1µg/ml to 4µg/ml (about 40% - 160% of the standard concentration of 0.5 mg/ml). Absorbance of these solutions were taken at 425nm using UV-visible spectrophotometer.

Curcumin content was calculated using the following formula:

It ranged from 74.98% to 85.03%. Maximum moisture was found in NDH-1 (85.03%) followed by NDH-3 (79.33%), Prabha (75.03%), NDH-2 (75.00%) and the lowest moisture content was found in NDH -98 (74.98%). Lokhande *et al.* (2013) [15] observed no significant difference in the moisture content of different Indian cultivars (Salem, Krishna and Tekurpetha).

Table 1: Moisture content

Sample Name	Moisture Content
NDH-1	85.03
NDH-2	75.00
NDH-3	79.33
NDH-98	74.98
PRABHA	75.03

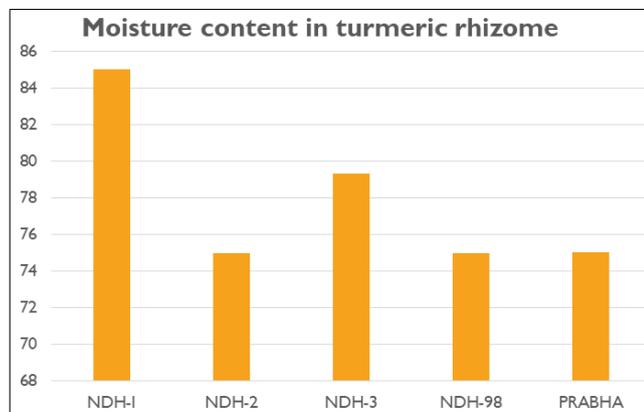


Fig 1: Moisture content

Total mineral content in turmeric rhizome

Data pertaining to total mineral content shown in Table-2 and Fig. - 2. It was revealed from data that total mineral content varied with variety to variety. It ranged from 7.01±0.34 to 12±0.34%. Maximum total mineral was found in NDH-1 (12±0.34%) followed by NDH-98 (11±0.34%), NDH-2 (9±0.34), NDH-3 (9±0.34%) and the lowest mineral content was found in Prabha (7.01±0.34%).

Table 2: Total mineral content

Sample Name	Total Mineral Content (%)
NDH-1	12±0.34
NDH-2	9±0.34
NDH-3	9±0.34
NDH-98	11±0.34
PRABHA	7.01±0.34

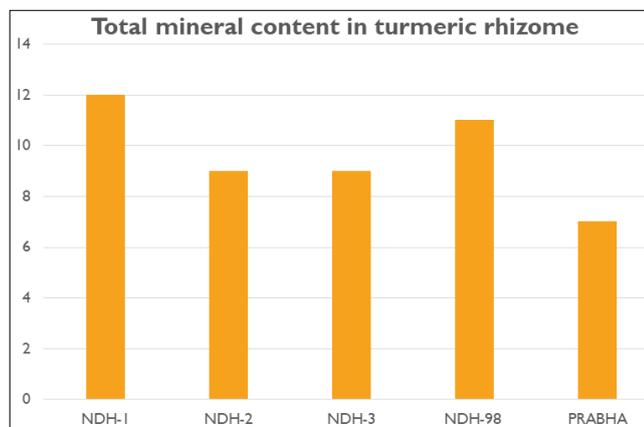


Fig 2: Total mineral content

Carbohydrate content in turmeric rhizome

Data pertaining to Carbohydrate content (%) shown in Table-3 and depicted in fig.-3. It was revealed from data that Carbohydrate content varied with variety to variety. It ranged from 61.50 to 70.40%. Maximum Carbohydrate was found in

NDH-2 (70.40%) followed by NDH-98 (70.10%), Prabha (66.30%), NDH-1 (64.10%), and the lowest Carbohydrate content was found in NDH-3 (61.50%). The result is in close favour with Lokhande *et al.* (2013) [15] who reported that carbohydrate content of all cultivars was in the range of 67.9 to 69.9 per cent.

Table 3: Carbohydrate content

Sample Name	Carbohydrate content (%)
NDH-1	64.1
NDH-2	70.4
NDH-3	61.5
NDH-98	70.3
PRABHA	66.3

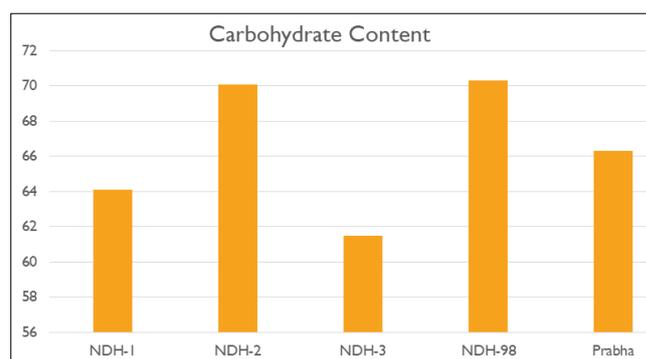


Fig 3: Carbohydrate content

Crude Fiber (%) in fresh Rhizome

Data pertaining to Crude fiber content (%) shown in Table-4 and depicted in fig.-4. It was revealed from data that Crude fiber content varied with variety to variety. It ranged from 4.64 to 5.65%. Maximum Crude Fiber (%) in fresh Rhizome was found in NDH-2 (5.65%) followed by NDH-98 (5.6%), NDH-3 (4.75%), NDH-1 (4.65%) and the lowest crude fiber content was found in Prabha (4.64%). A similar observation was also recorded by Lokhande *et al.* (2013) [15] who reported that crude fibre content of all cultivars was in the range of 7.19 to 8.06 per cent and maximum value was in Tekurpetha followed by Salem and Krishna.

Table 4: Crude fiber content

Sample Name	Fiber Content (%)
NDH-1	4.65
NDH-2	5.65
NDH-3	4.75
NDH-98	5.60
PRABHA	4.64

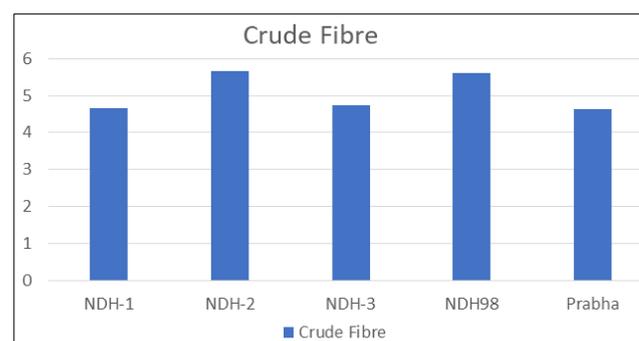


Fig 4: Crude fiber content

Oleoresin content

In respect of data on oleoresin content given in table-5 and illustrated in fig-5, indicate that oleoresin content varied with variety to variety. It ranges from 5.7% to 21.9%. Data further reveals that maximum oleoresin content was found in NDH-2 (21.9%) followed by Prabha (15.4%), NDH -1 (11.7%), NDH-3 (8.8%) and the lowest oleoresin content was found in NDH- 98 (5.7%). The result was closely supported by Sasikumar *et al.* 1996 [25] who observed that IISR varieties Prabha and Pratibha had 15.0 to 16.2% oleoresin content respectively. Lokhande *et al.* 2013 [15] reported significant difference in all the recent three cultivars.

Table 5: Oleoresin content

Sample Code	Oleoresin Content (mg/gm)	Oleoresin Content (%)
NDH-1	117	11.7
NDH-2	219	21.9
NDH-3	88	8.8
NDH-98	57	5.7
Prabha	154	15.4

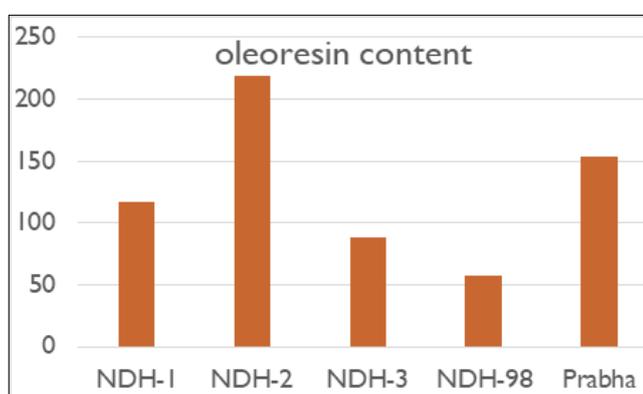


Fig 5: Oleoresin content

Curcumin content in turmeric rhizome

Curcumin, the most active polyphenolic constituent of turmeric curcuminoids obtained from rhizome *Curcuma longa* holds a high place in Ayurvedic medicine. It has been shown that curcumin have a wide spectrum of biological activities such as antifungal, antidiabetic, antioxidant, anti-inflammatory, anti-cancer, anti-allergic, anti-protozoal and antibacterial activities.

Data on curcumin content have been shown in Table: 6 and depicted in fig: 6. It was revealed from data that curcumin content varied with variety to variety. It was ranged from 2.22% to 8.44% among five varieties of turmeric. The maximum curcumin content was recorded in NDH-2 (8.442%) followed by NDH-1 (7.715%), NDH-98 (7.626%) Prabha (6.733%) and minimum percentage was found in NDH-3 (2.248%). The result was in close agreement with Krishnamurthy *et al.* (1975) [9], Kumar *et al.* (1997) [10] and Fatterpurkar *et al.* (2009) [4]. Sasikumar *et al.* (1996) [25] observed that IISR Prabha and Pratibha had 6.25 and 6.21 per cent curcumin content, respectively. Whereas, highest range of curcumin percentage was 3.584 to 7.730% in Pratibha followed by Salem 2.169 to 5.932%, Rajapuri 2.812 to 4.366%, Krishna 1.599 to 3.520% respectively, as observed by Kamble *et al.*, 2011. Factors such as the extraction method, solvent used for extraction, extraction time and temperature, the solvent ratio and extraction pressure were among the significant factors that were shown to be able to influence the

efficiency of curcumin extraction (Wakte *et al.*, 2011) [30]. Curcumin content was reported to vary from one species to another. Several studies have shown that soil factors, including nutrients and level acidity as well as the genus diversity, may affect the content of curcumin (Nahak and Sahu, 2011) [17].

Table 6: Curcumin content

Sample code	Curcumin Content (mg/gm)	Curcumin Content (%)
NDH-1	77.15	7.72
NDH-2	84.42	8.44
NDH-3	22.48	2.25
NDH-98	76.26	7.63
Prabha	67.33	6.73

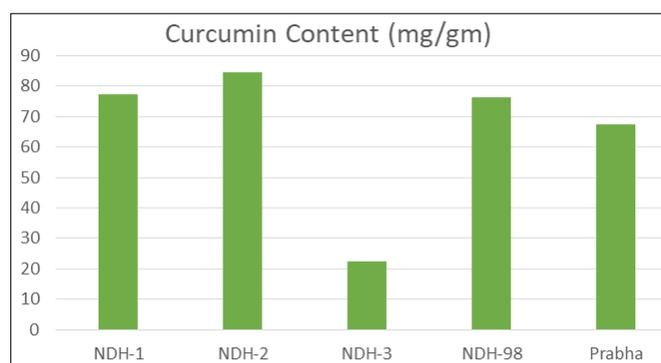


Fig 6: Curcumin content

Extraction of essential oil (Hydro distillation for isolation of essential oil)

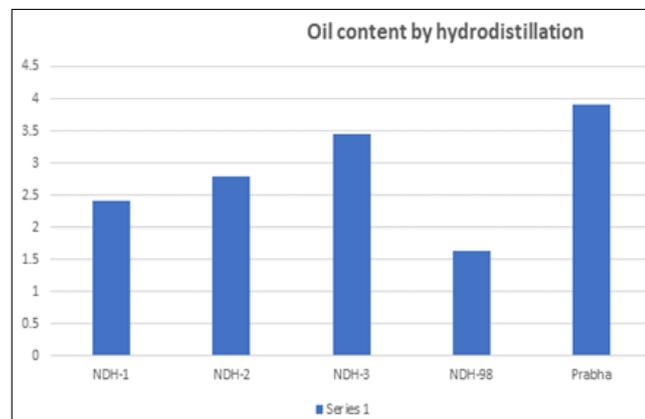
Data pertaining to essential oil content in turmeric rhizome have been given in Table:7 and depicted in Fig:7, indicated that essential oil content varied from variety to variety and it ranged from 1.63 to 3.91%. Data further reveals that maximum essential oil content was found in Prabha (3.91%) followed by NDH-3 (3.45%), NDH-2 (2.79%), NDH-1 (2.42%) and the lowest in NDH-98 (1.63%).

Results are in close agreement with text of Krishnamurthy *et al.* (1975) [9], Sasikumar *et al.* (1996) [25] and Kumar *et al.* (1997) [10]. Corray *et al.* (1988) [3] reported that optimum time for harvest for maximum yield of turmeric oil was 7.0 to 8.0 months and oil content in bulb was higher than that of finger rhizome. The roots yield highest concentration of oil 4.3 percent followed by rhizome 3.8 percent (Leela *et al.*, 2002) [12]. Volatile oil content in dried powder of *C. longa* rhizome was investigated by hydrodistillation and average yields of volatile oil in dried turmeric powder were $8.20 \pm 1.66\%$ v/w (Singh and Jain, 2011) [27]. There were significant variations in the composition of essential oils of turmeric rhizomes with varieties and geographical locations. Turmeric oil from different sources may have different chemical profile with different bioactivities. However, the production of these bioactive compounds depends on plant genotypes, postharvest processing (eg. Drying, extraction etc.), environment condition such as temperature, humidity, light, soil and geographical location (Li *et al.*, 2011) [13].

Plant maturity has also significant impact on chemical composition of turmeric oil. Both total curcuminoids and curcumin in rhizome reach the highest yield at 5-6 months and maturity result in decline of these pigments but the essential oils will not reach maximum yield until 7-10 months (Cooray *et al.*, 1988) [3].

Table 7: Essential oil content

S. No.	Sample	Oil content
1.	NDH-1	2.42%
2.	NDH-2	2.79%
3.	NDH-3	3.45%
4.	NDH-98	1.63%
5.	Prabha	3.91%

**Fig 7:** Essential oil content

Summary

Moisture content ranged from 74.98% to 85.03% and maximum moisture was found in NDH-1 (85.03%). Total mineral content ranged from 7.01 ± 0.34 to 12 ± 0.34 % and maximum total mineral content was found in NDH-1 (12 ± 0.34 %). Crude fiber content varied with variety to variety. It ranged from 4.64 to 5.65%. Maximum Crude Fiber (%) in fresh Rhizome was found in NDH-2 (5.65%) followed by NDH-98 (5.6%), NDH-3 (4.75%), NDH-1 (4.65%) and the lowest crude fiber content was found in Prabha (4.64%). Carbohydrate content ranged from 61.50 to 70.40% and maximum Carbohydrate was found in NDH-2 (70.40%). curcumin content varied from (2.22%) to (8.44%) among five varieties of turmeric maximum curcumin content was recorded in NDH-2 (8.442%). Oleoresin content ranges from 5.7% to 21.9% and maximum content was found in NDH-2 (21.9%). Essential oil content ranged from 1.63 to 3.91%. Data further revealed that maximum essential oil content was found in Prabha (3.91%) followed by NDH-3 (3.45%), NDH-2 (2.79%) NDH-1 (2.42%) and the lowest in NDH-98 (1.63%). The Turmeric essential oil of variety NDH-3 showed a highest effect in inhibiting DPPH, reaching up to 94.59% at concentration 50 μ l followed by NDH-2 89.96%.

Conclusion

The results obtained from the analysis of fresh turmeric rhizome indicated that maximum moisture was found in NDH-1 (85.03%). maximum total mineral content was found in NDH-1 (12 ± 0.34 %), Maximum Crude Fiber (%) in fresh Rhizome was found in NDH-2 (5.65%), maximum Carbohydrate was found in NDH-2 (70.40%), maximum curcumin content was recorded in NDH-2 (8.442%), maximum content was found in NDH-2 (21.9%), while highest essential oil content in Prabha (3.91%) among all five varieties.

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