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# Influence of ginger cultivars and maturity stages on oleoresin, 6-gingerol, polyphenol contents and antioxidant property

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*Zingiber officinale* Rosc, commonly called Ginger, is an underground rhizome widely used as a spice in the food and beverage industries. The prominent bioactive component of ginger is [6]-gingerol. The present study focuses on influence of the variety and maturity stage of ginger on [6]-gingerol content. Ten authentic cultivars of ginger were collected from ICAR-Indian Institute of Spice Research, Calicut, Kerala, India and analyzed for oleoresin, [6]-gingerol, polyphenol and antioxidant activity. Among these varieties, ING 5 variety gave the maximum oleoresin yield (11.05%) followed by ING 6 (10.5%). The [6]-gingerol content (7.59%) and the total polyphenol content (TPC) and antioxidant activity (85±0.5% at 200 ppm) were maximum in ING 6. Among these varieties, ING 6 was cultivated in Mysore for maturity studies. From 150 to 270 days of planting fresh ginger rhizomes showed oleoresin in the 6-10% range and [6]-gingerol was 2.26- 7.28%. Volatile oil (1.03± 0.2%) content did not show much change. With an increase in maturity, TPC and antioxidant activity also increased proportionally from 60 to 90% when compared to butylated hydroxyanisole (BHA).

**Keywords**: Ginger, Spices, *Zingiber officinale*

India, with its distinct agro-ecosystems differentiated by climatic conditions, soil, geological and vegetational features, is suitable for cultivation of a wide variety of crops. Ginger (*Zingiber officinale* Rosc,) rhizome is a prominent spice produced and exported from India. It has numerous applications as condiments, flavouring agent, and as therapeutic agent in pharmaceutical applications[1]. Ginger is an important spice crop of India covering 1.5 lakh ha area and contributing about 35%, to the world's total production[32]. India's annual production of ginger is 22.25 lakh tonnes (Fresah) for the year 2021, mainly grown in Kerala followed by Karnataka, Orissa, Meghalaya and Himachal Pradesh[3]. Generally, ginger is grown in moistened or rainfed areas, because of constant rainfall[4]. Nine month old-matured fresh ginger rhizomes have high moisture content resulting in low yield after drying. Farmers spend high amount of money for drying ginger. Drying makes ginger highly shrivelled and unattractive. Hence, it is marketed mostly as fresh green ginger[5].

Globally ginger is known for its medicinal value as it is effective for disorders of the gastrointestinal tract such as constipation, dyspepsia, diarrhoea, nausea and vomiting[6,7]. In South America, the homeopathy system recommends ginger for use in cardiopathy, high blood pressure, palpitation and as vasodilator[6]. Ginger is used as a major spice mainly for its unique taste and aroma present as essential oil and oleoresin[8]. Monoterpenes and sesquiterpenes in essential oil contribute to the specific flavour of ginger. The volatile oleoresin is responsible for the pungent taste of ginger, which is also a good source of antioxidant[9]. Essential oil and oleoresin are being used in the pharmaceutical and food processing industries.

Characterisation of essential oil and oleoresin from ginger cultivars from different geographical locations is the main area of investigation[10,11]. Govindarajan & Connell[5] have recorded the impact of climatic conditions on ginger rhizome growth and composition and showed that humid climatic conditions are favourable for growth. The extraction of oleoresin by solvent embodies pungency, and it comprises a group of non-volatile phenolics known as gingerols and Shogaols[12]. Wohlmuth *et al*. reported genetic variability in the essential oil content and amount of

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gingerol present in tetraploid and diploid clones of Australian ginger cultivars [13]. Bailey-Shaw *et al.* [14] demonstrated that the oleoresin and gingerol contents vary with maturation and environment. They also documented the changes in the contents of oleoresin and pungent compounds in Jamaican ginger during the 6-8 month maturity period. Investigation on the essential oil composition and oleoresin yield of fresh ginger cultivars of the north east region during 6-9 months maturity period have been reported[15]. Effect of variety, location & maturity stage at harvesting, on essential oil chemical composition, and weight yield of *Zingiber officinale* roscoe grown in Sri Lanka[16]. In many studies, the alcoholic extracts of several plants flowers, seeds, leaves, barks and agro-wastes have been demonstrated as good antioxidants for stabilizing vegetable oils[17,18].

The extraction of [6]-gingerol using efficient improved methods, and variation in the composition of extracts with respect to different stages of maturity including antioxidant properties, are not well demonstrated. The [6]-gingerol is one of the active compounds from ginger thant has significant antioxidative activity, but its content varies with variety and maturity stage. In the present study, we investigated the variation in oleoresin yield, the content of [6]-gingerol, total polyphenol content and antioxidant properties of 10 Indian commercial ginger cultivars collected from different locations in India, and further check for superior varieties and suggest appropriate maturity stage for harvest quality ginger.

## Materials and Methods

### Studies on varietal difference

*Sample Preparation*

The most popular ten commercial ginger cultivars including Mahima, Varada, Rejatha, Mahim, Maran, Rio-de-Janeiro, Aswathy, Athira, Karthika and Nadia were collected from ICAR-IISR Calicut, India (Table 1). All harvested samples were washed and air-dried. Fresh ginger was sliced (size 30 mm) using a slicer and spread uniformly on perforated trays for drying. The sliced ginger was then dehydrated in a hot air drier at 55±2ºC for 8 h. The dried ginger slices were powdered using a comminuting mill and stored at 4°C until further analysis. The moisture content of fresh and dehydrated ginger was determined using the toluene co-distillation method in duplicate according to AOAC[19] procedures.

Table 1 — Ginger cultivars and their origin procured from ICAR-IISR Calicut, Kerala, India

| Variety | Source of origin | ID (Indian Ginger) |
|---|---|---|
| Mahima | ICAR-IISR Calicut | ING 1 |
| Varada | ICAR-IISR Calicut | ING 2 |
| Rejatha | From ICAR-IISR Calicut | ING 3 |
| Mahim | Local cultivar from Maharashtra | ING 4 |
| Maran | Local cultivar from Kerala | ING 5 |
| Rio De Jeneiro | Local Variety from Karnataka | ING 6 |
| Aswathy | Kerala Agriculture University | ING 7 |
| Athira | Kerala Agriculture University | ING 8 |
| Karthika | Kerala Agriculture University | ING 9 |
| Nadia | Local cultivar from North East | ING 10 |

*Extraction*

Dried ginger slices were ground and passed through 500 µm size mesh, and this was used for all extractions. A glass column was loaded with dehydrated ginger powder steeped in ethanol (1:10) and allowed to stand for 2 h and then eluted. The eluted extract was distilled using flash evaporator at 50ºC under reduced pressure (40 millibars) and stored at 4ºC[20].

$$\text{Total extract content (W)} = \text{W2} - \text{W1}$$

where, W1 = Weight of the empty dried flask; W2 = Weight of flask with desolventised ginger extract.

*Analysis of [6]-gingerol by TLC*

The [6]-gingerol was estimated by thin-layer chromatography (TLC), Rf value was calculated, and quantitative study was carried out by UV-Spectrophotometer method[21].

*Preparation of sample*

Ginger oleoresin (0.05 g) was dissolved in 0.5 mL of diethyl ether. TLC chamber was filled with hexane and diethyl ether in the ratio of 1:1.5 (40:60) which has been thoroughly saturated with the solvent vapours. Ten µL of the sample were spotted to the 20 × 20 cm silica gel TLC plates (Merck, Germany) after dividing the plate into six equal parts. The plate was exposed for 5 to 10 min to drive off the solvent and sprayed with Folin-Denis reagent. Two major blue spots developed, the spot with higher Rf (0.54 to 0.71) being waxes and colouring matter and spot with the lower Rf value (0.26 to 0.29) being [6]-gingerol. The area representing [6]-gingerol was scooped with a small stainless-steel spatula by encircling the spot as well as 0.1 to 0.2 cm away from the actual spot. Three mL of distilled water was added and shaken well in a test tube. One mL Folin-Denis reagent was pipetted into the tube and mixed well. After 3 to 4 min, 1 mL saturated NaCO3 was added, mixed thoroughly for 5 min and kept for 1 h. A reagent blank was prepared with approximately the same amount of all reagents without a sample to serve as a blank. The optical density (OD) was read in a UV-spectrophotometer (UV-1800) Shimadzu at 725 nm. The amount of [6]-gingerol was calculated against the standard graph derived using vanillin at different concentrations.

$$\%\ [6]\text{-gingerol content} = (X \times 100)/Q$$

where, X is graph reading for gingerol in 1 mL solution in µg and Q is the quantity of extract in a spot

*Analysis of [6]-gingerol by HPLC*

Quantitative analysis of 6-gingerol was carried out by HPLC[22]. Fifty mg of ginger extract was dissolved in 2 mL of acetone (AR grade). Ten µL were injected into the HPLC using a reversed phase C-18 column, having particle size 3 µm and 4.6×250 mm. The detector was used with 2485 dual wavelength, water: ethanol: acetonitrile (52:5:43) as the mobile phase. The HPLC parameters were: flow rate 2 mL $min^{-1}$, consisting of model 515 pumps the eluting compound for the detection at the maximum wavelength of 280 nm.

*Total polyphenols*

Folin–Ciocalteau colourimetric method[23] was followed to determine total polyphenol content (TPC) of ginger extracts. One mL of the sample containing 1 mg of dry ginger extract, FC reagent (0.5 mL) and 20% saturated sodium carbonate solution (1.5 mL) were added. The mixture was made up to 10 mL with distilled water. UV-visible spectrophotometer was used to measure absorbance at 765 nm, after 60 min of incubation at room temperature. Results are expressed as mg of gallic acid equivalent per g of the extract (mg GAE $g^{-1}$).

*Radical scavenging activity*

Radical scavenging activity (RSA) was done for ginger extract[24] with slight modifications using DPPH. Different concentrations of the extracts from the varieties were taken in different test tubes and made up to 1mL by using distilled water. Four mL of DPPH was added to the test tubes, mixed and allowed to stand at 27ºC for 20 min in the dark. The absorbance of the samples was measured at 517 nm against control. RSA was calculated using the formula,

$$\text{RSA\%} = \text{Control OD} - \text{Sample OD}/\text{Control OD} \times 100$$

**Influence of maturity on gingerol content**

"ING 6" variety was used for maturity studies because of its higher [6]-gingerol content and antioxidant activity. This variety was grown in Mysore in April 2017. After an initial growth period of 5 months, fresh rhizomes (10 plants) were harvested every month continuously for 5 months and analyzed for [6]-gingerol, TPC content and antioxidant activity. Fresh rhizomes were analyzed for essential oil every month by hydrodistillation method. Ginger oil collected was dried with anhydrous sodium sulphate and stored at –4ºC for further GC-MS analysis [25].

## Results and Discussion

All studies were carried out in triplicate. The mean moisture content of selected 10 cultivars varied between 9 and 11 %. Van Beek *et al.*[26] reported the moisture content of Vietnamese ginger ranged from 8.7 to 10%. In the present study, oleoresin yield ranged between 5 and 11% (Fig. 1) among the 10 cultivars ING 5 yielded the highest (11±0.5%) amount of oleoresin followed by ING 6 (10 ±0.5%) and lowest (5.5%) oleoresin content was in ING10 variety. In earlier studies, some variations were noted concerning the locations, as the oleoresin content of IISR Mahima and IISR Rejatha were 4.48% and 6.64%, respectively[27].

**[6]-gingerol content by TLC and HPLC**

[6]-gingerol was isolated by TLC and quantified by UV spectrophotometric method . The results of [6]-gingerol yields from 10 cultivars are shown in Fig. 1. The [6]-gingerol content ranged from 3 to 8% in the 10 varieties tested among which ING 6 had the maximum gingerol (7.5±0.25) content followed by

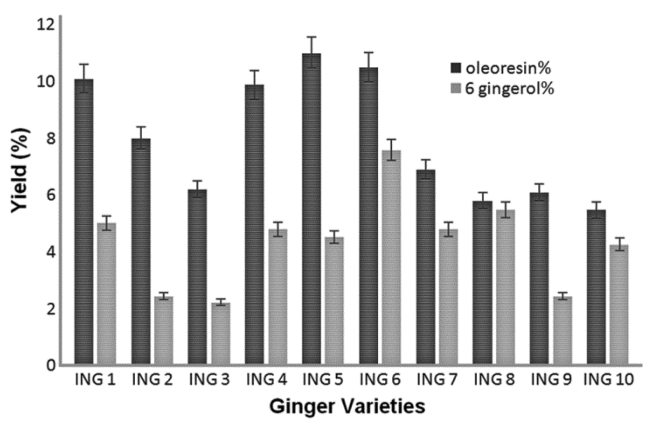


Fig. 1 — Oleoresin yield and 6-Gingerol concentrations with respect to selected 10 variants.

ING 8 (5.48±0.15). HPLC profile confirmed the presence of [6]-gingerol, and its analogues such as [8]-gingerol, [10]-gingerol and [6]-shogoal as seen in Fig. 2. Similar results were reported that the amount of gingerol recovered from oleoresin samples from seven locations ranged between 1.39and 6.72%[28].

**Total polyphenol content (TPC) and Radical scavenging antioxidant activity**

The TPC of ginger extract from each cultivar is presented in Fig. 3. It was also clear that ING 6 had the maximum total polyphenol content (36±2 mg $g^{-1}$) followed by ING5 (32.3±0.6) whereas, the least TPC was observed in ING1 (19.6±0.2). Earlier report[29] has shown that the phenolic and flavonoid compounds present in plant extracts are responsible for antioxidant activity.

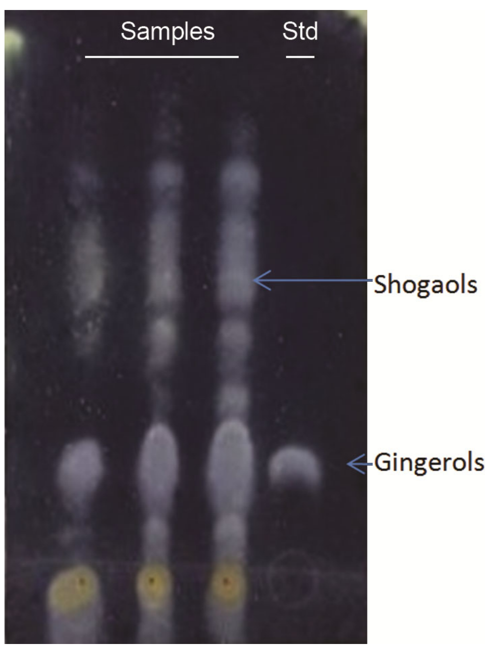


Fig. 2 — Thin Layer Chromatography of gingerols and Shogaols

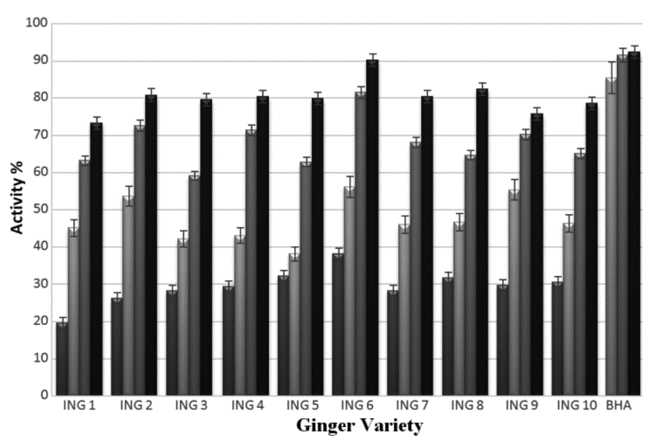


Fig. 3 — Free radical scavenging activity of 10 selected Ginger verities

DPPH radical scavenging activity ranged from 85-92% at 200 ppm (Fig. 3). ING 6 exhibited maximum antioxidant activity (91.12 %) followed by ING5 (80.3%) and the lowest antioxidant activity was in ING1 (73±2%). Earlier Pawar *et al.*,[28] showed that the antioxidant activity, total phenolic, and [6]-gingerol contents varied considerably from cultivar to cultivar Recently, Tebboub & Kechrid[30] reported that ginger has a powerful effect, which led to a reduction of diabetes development in zinc deficiency due to its antioxidant potential.

**Maturity studies**

Among the 10 selected cultivars, ING 6 was selected for conducting maturity study in Mysore because of its highest bioactive components. After 150 days of sowing, every 30 days interval rhizomes were harvested, washed, and analyzed for biomass weight. The cultivar (ING 6) showed a gradual increase in weight of rhizome (200 to 700 g) with increasing maturity (Fig 4A), but the weights of leaves (29 to 12 g), stem (72 to 24 g) and flowers (18 to 11 g) were gradually decreasing.

After controlled drying of fresh ginger, it was powdered and used for oleoresin extraction. The percent of non-volatile compounds such as gingerols were quantified and estimated by TLC and HPLC; results are shown in Fig 4B and Table 2. It can be observed that [6]-gingerol content and oleoresin yield gradually increased from 2 to 7% and 6 to 10%, from 5 to 10 months, respectively. The increase was also observed in TPC content (14.54 to 35.86%) and antioxidant activity (85 to 92%) as it reached the maturity stage (Fig 5). However, ginger oil yield did not show a great increase (1.02±0.2) from 150 to 270 days. Major volatile compounds identified in fresh ginger rhizome were zingiberene, sequiphellandrene,

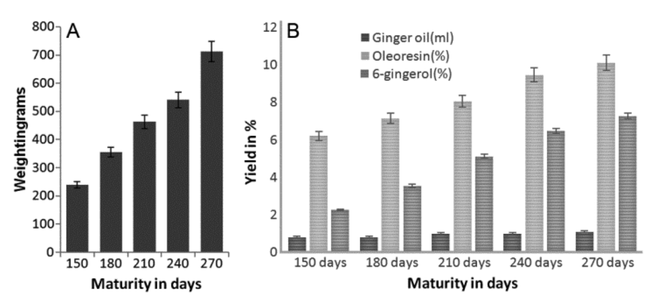


Fig. 4 — (A) Rhizome weight of ING6 variety; and (B) Yield of Ginger oil, oleoresin and 6-Gingerol content with respect to Maturity

Table 2 — HPLC profile of selected ING 6 verity with respect to 6-gingerol against maturity

| Maturity (in days) | Retention Time (min) | Area concentration (% ) |
|---|---|---|
| 150 | 3.920 | 21.7 ±0.5 |
| 180 | 3.900 | 34.6 ±0.8 |
| 210 | 3.890 | 37.3 ±0.3 |
| 240 | 3.894 | 42.8 ±0.6 |
| 270 | 3.893 | 49.4 ±0.7 |

[Rhizomes were harvested during August 2017 to December 2017 for maturity studies]

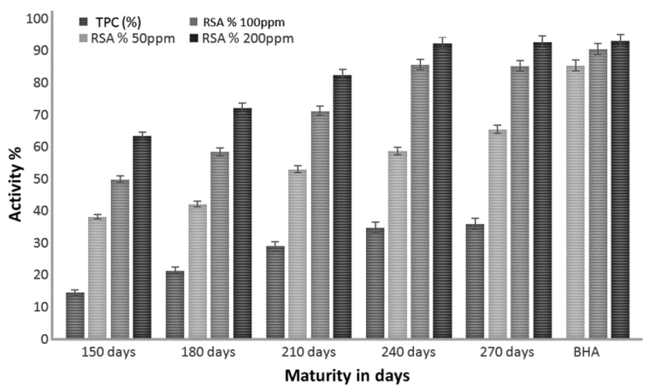


Fig. 5 — Free radical scavenging activity of ginger extracts with increasing maturity

ar-curcumin, sesquiterpene. The volatile oil composition depends upon the maturity and growing location[15]. Studies carried out by Natarajan *et al.*[29] with twenty-six varieties of ginger showed that the volatile oil and ginger extracts increased steadily after the fifth month. The quality and quantity of metabolites in plants responsible for biological activities are influenced by a multitude of factors, chief among them, environmental[31]. Kiran *et al.*[32] reported that variation in the essential oil composition and oleoresin and [6]-gingerol contents in 10 different fresh ginger cultivars harvested at 6- and 9-month maturity from five different states of NER. Monoterpenes, sesquiterpenes, and citral composition in the essential oil were evaluated to ascertain their dependence upon the maturity of ginger. Present study provided a comprehensive analysis in essential oil composition, weight of rhizome, oleoresin yield, [6]-gingerol content and radical Scavenging activity of ING 6 with increasing maturity (150 to 270 days). ING 5 had a higher amount of oleoresin and ING6 and ING 8 had a higher content of 6-gaingerolwhich is unique among 10 popular cultivars in South India and provides valuable information to the flavor industry to choose these cultivars with the desired aroma profile.

## Conclusion

The quality and quantity of metabolites in ginger responsible for biological activities are influenced by a multitude of factors. Among the ten selected ginger cultivar, only ING5 gave the highest yield of ginger oleoresin (11.05%) followed by ING6 (10.5%). [6]-gingerol content was maximum in ING 6 (7.5±0.5%). It can be concluded that ING 6 cultivar with utmost [6]-gingerol content possesses maximum free radical scavenging activity. The rhizome weight which increased from 200 to 700 g could be recommended for cultivation to get improve their harvest at maturity. It would be interesting to investigate the genotypes like Rio De Janeiro which have high 6-gingerol content.

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## Conflict of Interest

Authors declare no conflict of interessts.

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