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Comparative Study of Extracts with Respect to Their Polyphenol Contents and Antioxidant Activity, From Rhizomes of *Zingiber officinale* Having Variable Harvesting Times

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ABSTRACT
The scope of natural constituents in treating multiple ailments has emerged to greater extent in current years. The number of plant materials containing polyphenols, are used nowadays as therapeutic and mainly as antioxidants. The polyphenols mainly 6-gingerol, derived from the rhizomes of ginger (Zingiber officinale) have better antioxidant properties. The anti-inflammatory, analgesic and antiemetic activity is found to improve with increase in phenolic contents. The plant rhizomes are harvested at different times. In the current study we have selected the rhizomes harvested at three different times viz. 09, 12 and 15 months from the date of plantation. The extraction is carried out using acetone and further comparative study is conducted on three extracts. The Total Phenolic Contents (TPC) was determined in terms of Gallic Acid Equivalents (GAE) mg/100g and it was observed that the TPC increased with the longer harvesting times. The 6-gingerol contents were estimated by RP-HPLC and found to improve with longer harvesting times. The quenching or inhibition activity of extracts on DPPH radicals were compared using fixed reaction time method to assess their antioxidant property. The antioxidant properties also improved with longer harvesting times. The overall comparative study concludes the longer harvesting times be the best for improved contents and activities of the ginger extracts. The futuristic study may be carried out to optimize the harvesting time of ginger rhizomes. Even the effect of post-harvesting storage time and storage conditions also needs to be conducted for the said plant.
KEYWORDS: Ginger, TPC, antioxidant activity, RP-HPLC, DPPH, harvesting time

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INTRODUCTION

The spice of India, Ginger (*Zingiber officinale*), being the member Zingiberaceae family, has a fresh aroma and pungent taste. It has been popular from ancient era as a remedy for number diseases. The Ayurveda has always highlighted the magic of the plant. Number of herbal formulations is in existence due to its broad range of medicinal characteristics. It is made up of many phenolic ketones. The primary component of ginger is gingerols, which are found in the rhizome. Among the gingerols found in fresh ginger rhizomes, 6-gingerol [5-hydroxy-1-(4-hydroxy-3methoxy phenyl) decan-3-one] is the most readily available variety [1]. Other gingerols include 4-gingerol, 8-gingerol, and 10-gingerol. Additionally, 6-gingerol exhibits anti-inflammatory, analgesic, and antioxidant qualities. Furthermore, 6-gingerol's anticancer properties have been demonstrated through experimentation [2]. Ginger has been shown to have antiemetic properties [2]. It has been demonstrated that 6-gingerol and ginger acetone extract are strong inhibitors of cyclophosphamide-induced vomiting in the suncus [4]. 6-gingerol's economic use in sectors including food, pharmaceutics, and agronomy depends on its antioxidant capacity. The dehydrated form of 6-gingerol, 6-shagol, is produced from the thermally unstable 6-gingerol [5]. To separate phytochemicals from plant materials, extraction is an essential step, and the extraction process affects the extract's quality. To optimize the extraction yield, various extraction techniques are applied. Among the several techniques are steam distillation, solvent extraction, and Soxhlet extraction [6]. Different technologies like microwave-assisted extraction (MAE), ultrasonic-assisted extraction, subcritical water extraction (SWE), and supercritical CO_2 fluid extraction are also used to extract gingerol

from the ginger rhizome [5,6]. Various isolation and purification techniques such as column chromatography, semi-preparative high-performance liquid chromatography and high-speed counter-current chromatography are used to purify 6-gingerol [7,8].
Generally, ginger is harvested in 8 to 9 months and can be extended up to 15 months from plantation. The present study compares the effect of harvesting time on Total Phenolic Contents (TPC), specially 6-gingerol one of the abundant gingerol present in ginger. Also, the antioxidant activity of extracts harvested at different times is also compared. The different harvesting times such as 9, 12 and 15 months are selected for the aforesaid comparative study.

MATERIAL AND METHODS

Preparation of Sample

The ginger rhizomes were directly collected from field as freshly harvested samples at the end of 9, 12 and 15 months from the known history of plantation provided by the farmer at *Alephata*. These were then sent to Department of Botany, Balasaheb Jadhav College of Arts Commerce and Science, Ale for authentication. The rhizomes were water washed to remove the dirt completely and cut into small fragments. These are then air dried for 21 and dried samples are then ground using the domestic grinder. The powdered samples are labeled as S1, S2 and S3 and preserved for further extraction [9].

Extraction method

About 100g of powdered sample is subjected to Soxhlet extraction using acetone as an extraction solvent. The extraction is carried out for 10 hours at about 80 ^{o}C temperature. The reminder powder is then washed with fresh acetone and the extract is concentrated. The solvent is then evaporated using rotary evaporator (Media make model EVII) under low pressure at about 40 ^{o}C temperature for 2 hours. The extract is then weighed and percentage yield of each batch is calculated using formula % yield=E/P X 100 and reported [10].

Chromatographic estimation of 6-gingerol

The estimation of 6-gingerol, the component of interest from the extract is carried out using High Performance Liquid Chromatographic System of made Shimadzu provided with Degassing unit DGU-20A5R, pump model LC-20AD and UV-VIS Detector SPD-20A. The study is conducted with following chromatographic conditions [11, 12, 13, 14]:

- Mobile phase: Acetonitrile: Water (60:40) (Isocratic Elution)
- Separation mode: Reverse phase
- Column: Shim-pack GIST C18 ODS, Size- 4.6 I.D. × 250 mm
- Flow rate: 1ml/min
- Temperature: 30^{o}C
- Detection wave length: 280nm

The calibration curve of peak area Vs concentration (ppm) was plotted and the concentration of 6-gingerol was estimated from the peak area of 6-gingerol from chromatogram of all three extracts.

Total Phenolic Content (TPC) Estimation

The total phenolic content was analyzed in extract by using F-C reagent test. It is determined and expressed as Gallic acid equivalent (GAE) in mg/100g of extract. The 10% w/v Folin Ciocalteu (FC) reagent was prepared in distilled water. The 7.5% w/v solution of sodium carbonate was prepared in distilled water. The Gallic Acid is used as a phenolic standard and its stock solution having concentration 0.1 mg/mL was prepared in methanol. It is then diluted to series of solutions having concentrations 0.02, 0.04, 0.06, 0.08 and 0.1mg/mL. 1 mL from each standard is added to a glass tube. 5mL of 10% w/v F-C reagent is added to it. Then after 5 minutes, 4mL sodium carbonate solution (7.5% w/v) is added to make it alkaline. The tubes are then shaken vigorously for a minute and incubated for one hour at room temperature in dark. The absorbance is measured at 760nm. The calibration curve is plotted as absorbance Vs known concentrations of Gallic acid. The same test is performed on 1mL of 1g extract in 10mL methanol solution (for all three extracts). The absorbance is measured at 760nm and the concentrations are determined from Gallic Acid calibration curve to determine Gallic acid equivalents in mg/100g [9].

Antioxidant Property (DPPH Assay)

The antioxidant activity of extract was determined as an ability of extract to scavenge the free radical DPPH (Diphenyl Picryl Hydrazyl). The stock solution of DPPH (100 μM) was prepared by dissolving 3.943 mg of DPPH in 100mL of 70% ethanol [15]. The fixed reaction time method was used [16]. In this method, 1.5 mL antioxidant solution (for three extracts and ascorbic acid as a positive control) is prepared in phosphate buffer (pH 7.5). The DPPH solution was added to the increasing concentration of ascorbic acid as a positive control and the absorbance was measured at 515nm after incubation for 30 minutes in dark.

The same procedure was adopted for extracts and the absorbance was measured to determine percent quenching (%Q) of DPPH. The %Q is calculated using formula, %Q = [(ADPPH – A sample)/ ADPPH] x 100.

RESULTS AND DISCUSSIONS

Extraction

The Soxhlet extraction using acetone gave light brown colored extracts having different percent yields depending on harvesting times as 11.60% for 9 months harvested sample (HT1), 12.80% for 12 months harvested sample (HT2) and 14.20% for 15 months harvested sample (HT3).

Chromatographic estimation of 6-gingerol

The calibration curve for standard 6-gingerol was prepared from chromatograms, as the peak area vs concentration (ppm) (Figure 5). The line with R^2 value 0.992 and equation y=41582x–185808 was obtained. The concentrations and then percent content of 6-gingerol in the extracts were found to be 2.13% (For HT1), 2.42% (For HT2) and 2.56% (For HT3) (Figure 1-4).

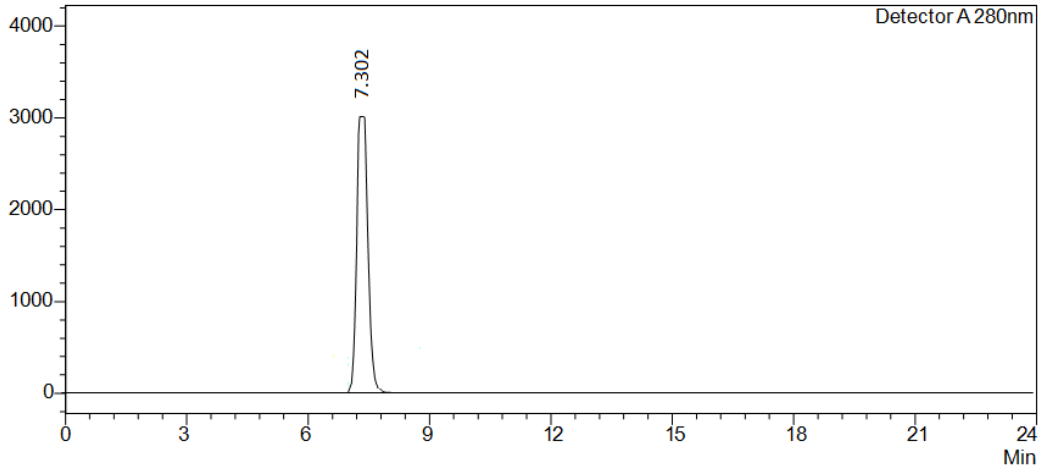

Figure 1: Chromatogram of Standard 6-gingerol

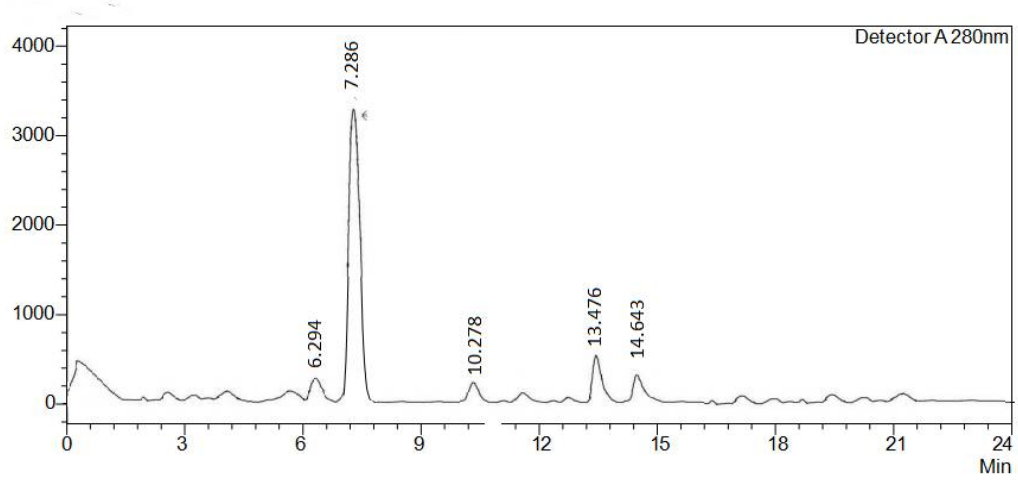

Figure 2: Chromatogram of Extract HT1

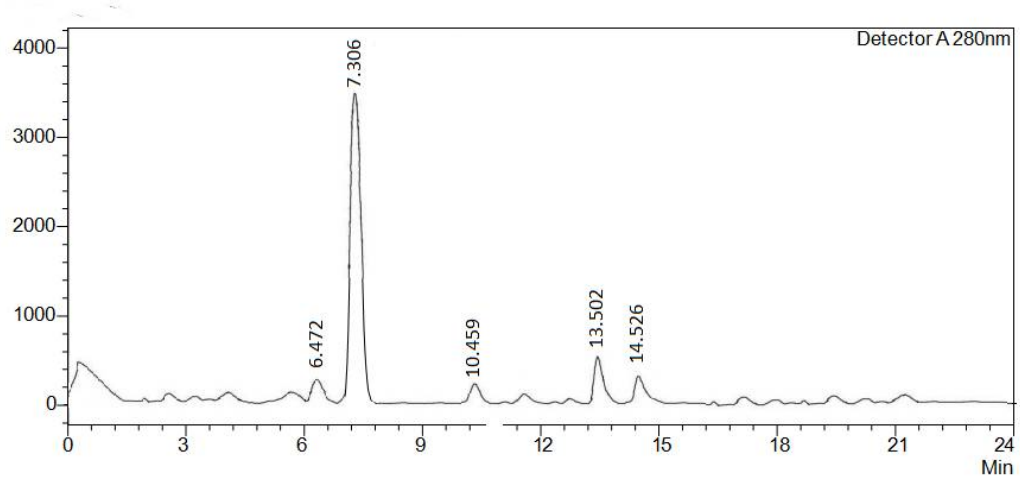

Figure 3: Chromatogram of Extract HT2

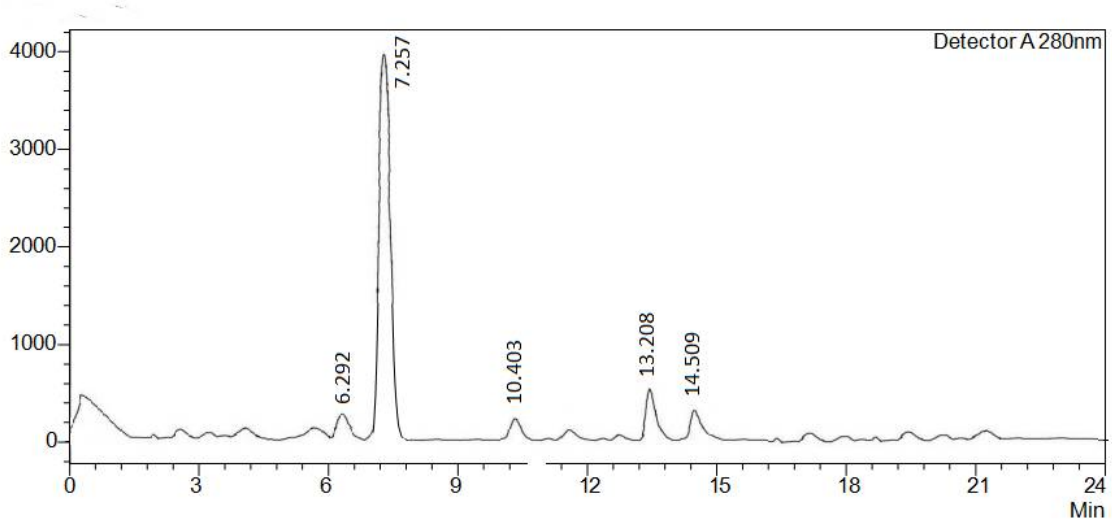

Figure 4: Chromatogram of Extract HT3

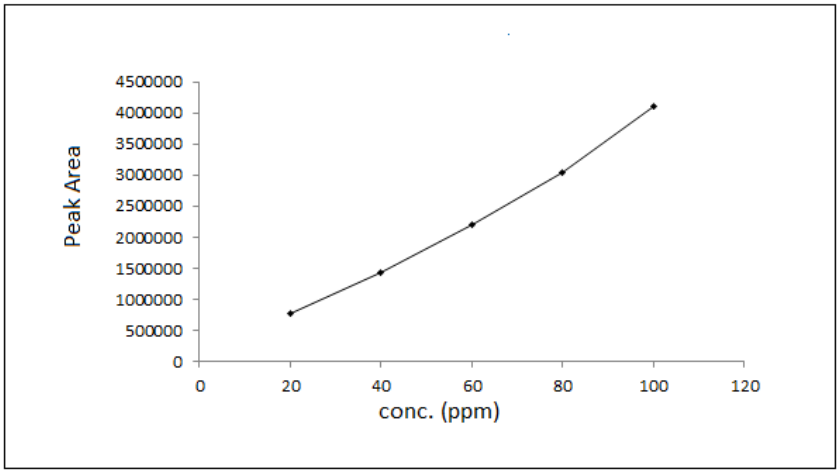

Figure 5: Calibration curve of Peak Area Vs Conc for 6-Gingerol

Total Phenolic Content (TPC) Analysis

The TPC of extracts were determined and expressed as Gallic Acid Equivalents mg/100g (Figure 6). The results for three different extracts were 155.00 GAE mg/100g (For HT1 extract), 187.00 GAE mg/100g (For HT2 extract) and 227.00 GAE mg/100g (For HT2 extract) (Figure 7).

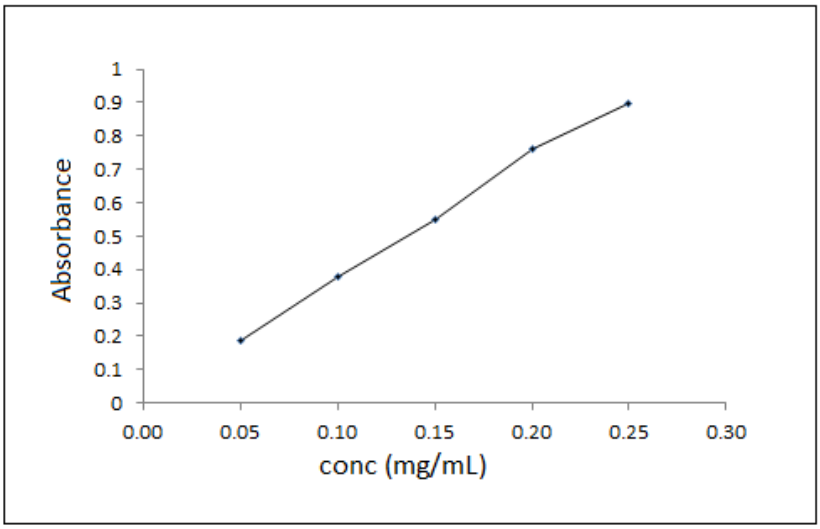

Figure 6: Gallic Acid Standard Curve

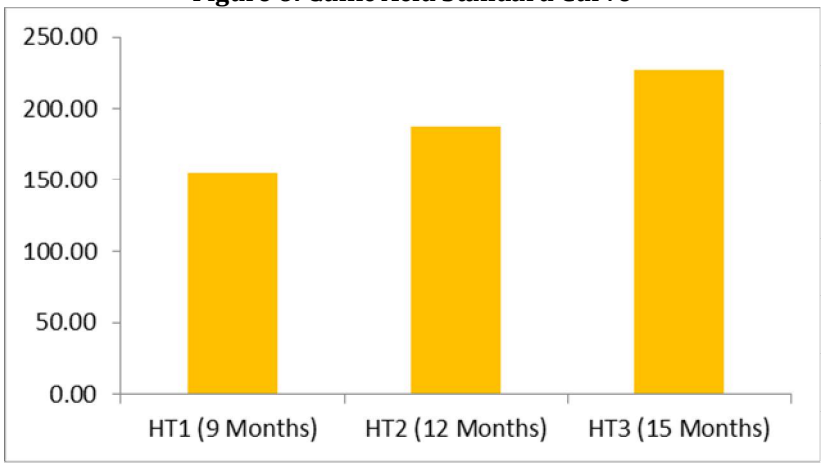

Figure 7: Total Phenolic Contents (GAE mg/100g)

Antioxidant Activity (DPPH Assay)

The percent quenching of DPPH radical is the direct measure of antioxidant activity of the compound. The fixed reaction time method with increasing concentrations of ascorbic acid (positive control) and three different extracts were compared (Figure 8) and it was found that the antioxidant activity of ginger extract improves with the harvesting time. The improved activity is the result of increased TPC with harvesting time.

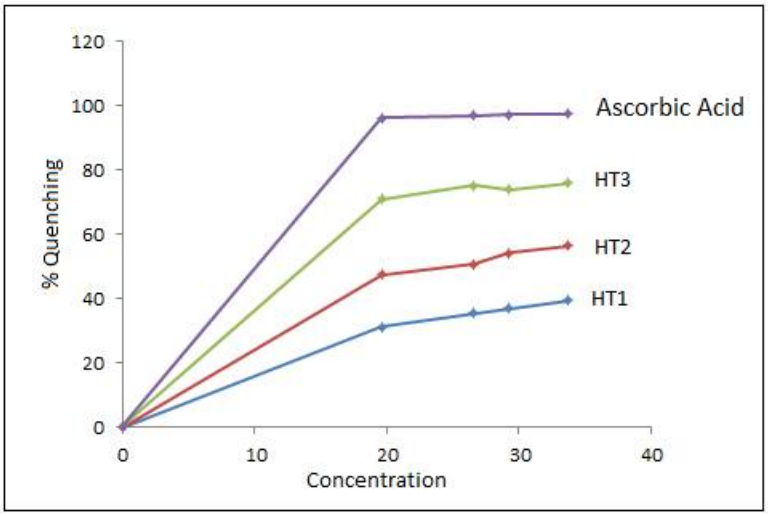

Figure 8: % Quenching of DPPH Radical Vs concentration

CONCLUSION

The ginger rhizome is the abundant source of phenolic compounds which exhibit medicinal properties against various ailments. The rhizomes are generally harvested between 8 to 9 months from the date of plantation. The present study reveals the importance of harvesting time for its futuristic use. The total phenolic contents and even 6-gingerol content improves with late harvesting. The antioxidant properties of the extracts also increase with the longer harvesting times. The signal to noise ratio of increased cost with longer harvesting times to improved contents shall be understood to select the optimum harvesting time of ginger rhizomes.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Mr. Sujit Tukaram Tambe has contributed in the selection of plant, harvesting and extraction of ginger rhizomes. Further procurement of chemicals and analytical study is also conducted by him. Being a supervisor Dr. Kaliyaperumal. Saravanan has guided the whole research work and the research work is conducted as per his suggestions and solutions.

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