

Characterization of 6-Gingerol for *In Vivo* and *In Vitro* Ginger (*Zingiber officinale*) Using High Performance Liquid Chromatography

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Abstract: Ginger (*Zingiber officinale* Rosco) belonging to the family Zingiberaceae is one of the world's most important spices and produces a pungent, aromatic rhizome that is valuable all over the world. Qualitative and quantitative analysis of 6-gingerol in different parts (*in vivo* and *in vitro*) of *Zingiber officinale* using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) have been performed. Data of TLC showed spots having identical R_f value (0.15), according to the synthetic standards of 6-gingerol in all samples extract. 6-gingerol was detected in all extracts of different parts of ginger derived from *in vivo* and *in vitro* culture conditions. Quantitative determination of 6-gingerol using HPLC technique was carried out. Comparing with the peaks of 6-gingerol in synthetic standards, *in vivo* rhizomes and *in vitro* cultures of different ginger parts was showed similar UV spectra characteristics. The quantity of 6-gingerol in rhizomes (*in vivo* and *in vitro*) and *in vitro* microrhizomes (45.37; 42.64; 28.11 mg/g respectively), were showed a higher value than that of *in vitro* calli, shoots and roots (7.89; 7.46; 6.40 mg/g respectively).

Keywords: Ginger, rhizome, *in vitro*, callus, TLC, HPLC.

INTRODUCTION

The use of herbs to alleviate pain and pestilence is as old as mankind itself, but it has now been dubbed as one of the alternative therapies in the allopathic system of medicine. Ginger (*Zingiber officinale* Rosco) belonging to the family Zingiberaceae is one of the world's most important spices and produces a pungent, aromatic rhizome that is valuable all over the world [1,2]. *Z. officinale* has been extensively studied for a broad range of biological activities. This species contains biologically active constituents including non-volatile pungent principles, such as the gingerols, shogaols, paradols and zingerone which produce the "hot" sensation in the mouth (Figure 1). Ginger has been increasingly used recently because of its low toxicity and its broad spectrum of biological and pharmacological applications [3, 12, 16, 17, 25]. The major oily constituents responsible for the pungent taste of ginger are homologous phenolic ketones known as gingerols [3]. In fresh ginger rhizome, gingerols were recognized as the major active components and 6-gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one is the main constituent in gingerol series [4, 5]. Similarly, paradol is formed on hydrogenation of shogaol [6]. Pungent and aroma compounds identification and quantitative analysis in

ginger have attracted the attention of researchers for many years. TLC is a routine method, which separates low molecular weight organic compounds according to their polarity. It is the most common technique used for the separation of natural substances [7]. HPLC has been considered to be a powerful tool for determination of gingerols in ginger. Moreover, Balladin and Headley [8] concluded that liquid chromatography method can be considered to be an appropriate method for the rapid identification and quantification of the essential oils and the pungent principles (shogaol and gingerol) of the extracted oleoresin from the solar dried ginger rhizomes. Therefore, qualitative and quantitative determination of 6-gingerol using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) for *in vivo* and *in vitro* cultures was conduct in this paper.

MATERIALS AND METHODS

Chemicals, Solvents and Authentic Reference Compound

Authentic Compound

6-gingerol (>98% purity) was purchased from Zhongxin Innova Laboratories, Tianjin Zhongxin Pharmaceutical Group Corporation Ltd, Avenue, TEDA, Tianjin 300457, China

Chemicals

Sodium sulphate anhydrous and sulphuric acid were purchased from Prolabo chemicals Co., St. louis

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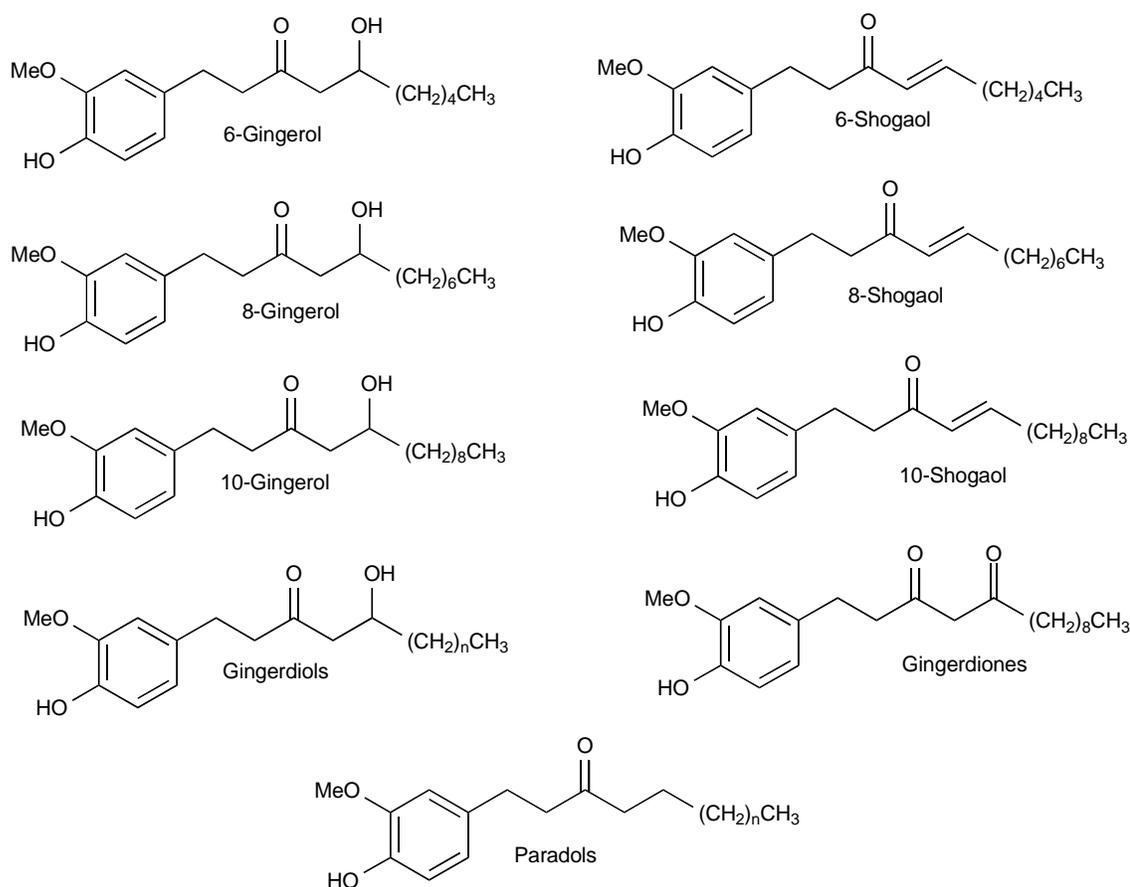


Figure 1: Chemical structure of the major pungent constituents of ginger.

Mo. USA, and silica gel 60 were purchased from Merck, Darmstadt, Germany.

Solvents

Acetone, Methanol, Ethyl Acetate, Diethyl Ether n-Hexane used was pure and analytical grade.

Extraction of Crude Extract

One gram of fresh ginger rhizome, *in vitro* root, *in vitro* shoot, callus, *in vitro* micro-rhizome and rhizomes derived from micropropagated plants under green house conditions [2] were dried using oven at 40°C then ground into a fine powder and kept in airtight bags at room temperature in darkness until used.

Samples transferred to a conical flask separately and percolated three times with methanol at room temperature and the methanolic extracts were combined, filtered, evaporated *in vacuo* at 45°C and divided into two groups kept with the residue obtained from the solvent extract at 4°C in sealed brown vials for analysis.

Methanolic residue was dissolved in ethyl acetate, shaken with water several times in a separating funnel

and partitioned between water and ethyl acetate and added to 10g sodium sulphate anhydrous to remove water content. The ethyl acetate layer was combined, filtered and concentrated to dryness *in vacuo* at 45°C . Residue was re-dissolved in methanol to analysis 6-gingerol by HPLC.

Thin Layer Chromatography (TLC)

The extracts and authentic reference were tested using TLC which performed on pre-coated silica gel 60 aluminum backing with (20x20 cm, layer thickness: 0.2 mm, Merck, Germany). TLC was carried out on plates, silica gel Merck, Darmstadt, Germany, 60 F₂₅₄, 20x20 cm.

Solvent systems: a mixture of n-hexane–acetone (8:2 v/v) as developing solvent according to the method described before [9].

Chromatogram development: a mixture of n-hexane–acetone (8:2 v/v) migrates the predetermined distance in the layer (silica gel Merck 60 F₂₅₄) by capillary action. In this process the samples are separated into fractions. After evaporation of the mobile phase the fractions remain stored on the layer. The

plate was sprayed with 10% sulphuric acid and heating on a hot plate to show bands according to the method by Hori *et al.* [10].

Chromatogram evaluation: The tracks are scanned with ultraviolet range of the spectrum. The bands of 6-gingerol in authentic reference and samples were calculated

High Performance Liquid Chromatography (HPLC)

Reverse-phase high performance liquid chromatography (HPLC) was used for the determination of 6-gingerol in the extract of ginger powders. Twenty μl of the extract was injected into the HPLC. The water HPLC system was used. The column was a reverse-phase HI5C₁₈ (150 mmx 4.6 mm), id 5 μm (Hichrom Co.) with Nova-Pak@ C₁₈ pre-column (Water Co.). The mobile phase was methanol: double de-ionized water (70:30 v/v). The solvent flow rate was 1.2 ml/min. The UV detector (Water™480) was monitored at 282nm according to the method described by Phoungchandang and Sanchai [11].

HPLC was carried out on Agilent a series 1100 interface applying the following conditions:

Stationary phase: HI5C₁₈

Injection volume: 5 μl

Oven temperature: Room temperature

Diode array detector: 282 nm

Flow rate: (1.2 ml / min)

Standard curves of authentic compounds and calculations of unknown amounts of 6-gingerol in the tissues were done using routine protocols as described in the "Instruction Manual for HSM Manager" as well as using the formula given by (Scott, 1996) [12]:

$$C(u) = A(u) \times C(st) / A(st)$$

Where: C (u) is the concentration of unknown sample.

A (u) is the peak area of the unknown sample.

C (st) is the concentration of the standard.

A (st) is the area peak of standard.

RESULTS AND DISCUSSION

Qualitative and quantitative determination of 6-gingerol were detected in *Zingiber officinale in vitro*

cultures and *in vivo* rhizomes. Two methods have been employed, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Thin Layer Chromatography (TLC)

TLC method was carried out as a preliminary screening to check the presence of 6-gingerol in different plant parts derived from *in vitro* cultures and *in vivo* rhizomes as shown in Table 1 and Figure 2. TLC analysis of this compound was compared with synthetic standards of 6-gingerol. Extracts of all samples showed spots having identical R_f value calculated according to the synthetic standards of 6-gingerol and the value of R_f was 0.15. Furthermore, 6-gingerol was detected in all extracts of different parts of plant derived from *in vitro* cultures and *in vivo* rhizomes. TLC is a standard technique, which separates low molecular weight organic compounds according to their polarity. It is the most common technique used for the separation of natural substances [7]. TLC is favored for its reliability and affordability [13].

Table 1: TLC Screening for the Presence of 6-Gingerol in Different Parts (In Vivo and In Vitro) of *Zingiber officinale*

Extracts	Detection of 6-gingerol
Synthetic standards (6-gingerol)	+
<i>In vivo</i> rhizomes	+
<i>In vitro</i> Rhizomes	+
<i>In vitro</i> shoots	+
<i>In vitro</i> roots	+
<i>In vitro</i> microrrhizomes	+
Calli cultures	+

(+) = detected.

Absorbent: Silica gel 60 (Merck).

Solvent: n-hexane-acetone (8:2 v/v).

Likewise, this technique was found to be useful in validating the identity of 6-gingerol by several authors [14-19]. On other hand, He *et al.* [9] reported that, the pungent compounds can be separated from ginger as groups by TLC on silica gel plates, but individual components cannot be well resolved.

High Performance Liquid Chromatography (HPLC)

Quantitative determination of 6-gingerol using HPLC method was carried out. Data presents in Figure 3A, B, C, D, E, F, and G showed that, peaks of 6-gingerol which detected in synthetic standards, *in vivo* rhizomes and different ginger parts derived from *in vitro* cultures

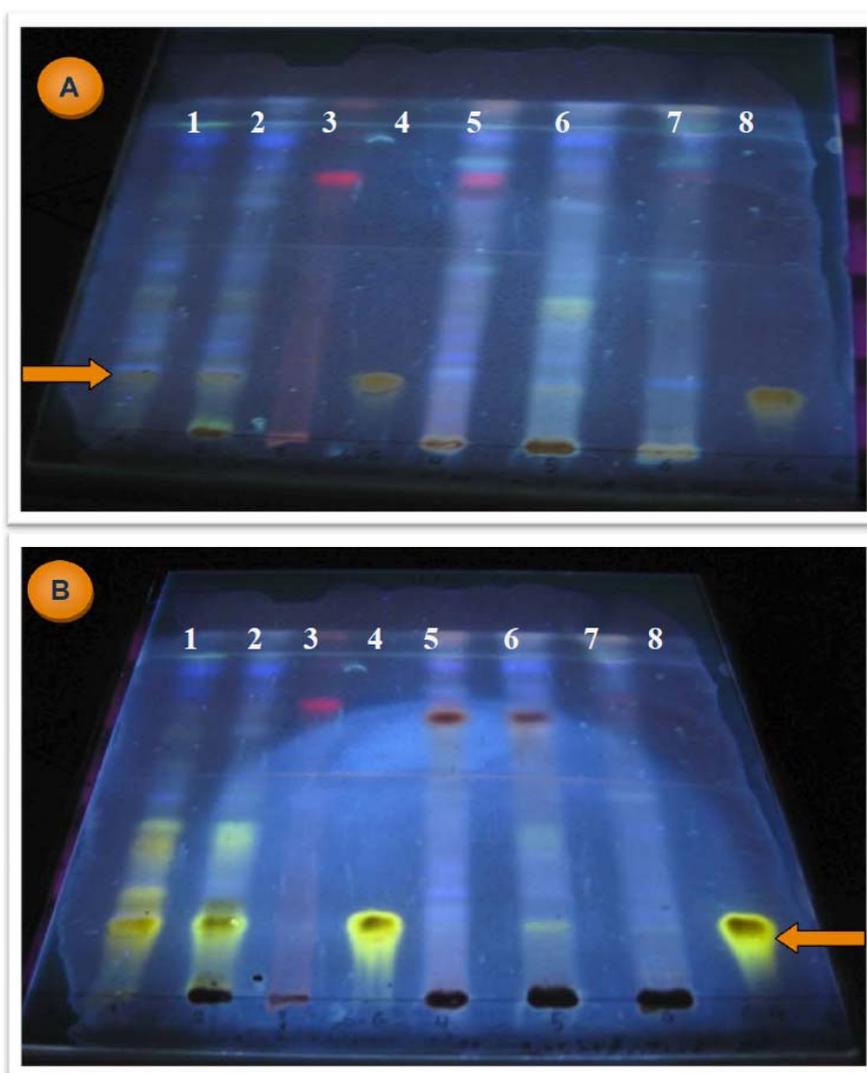
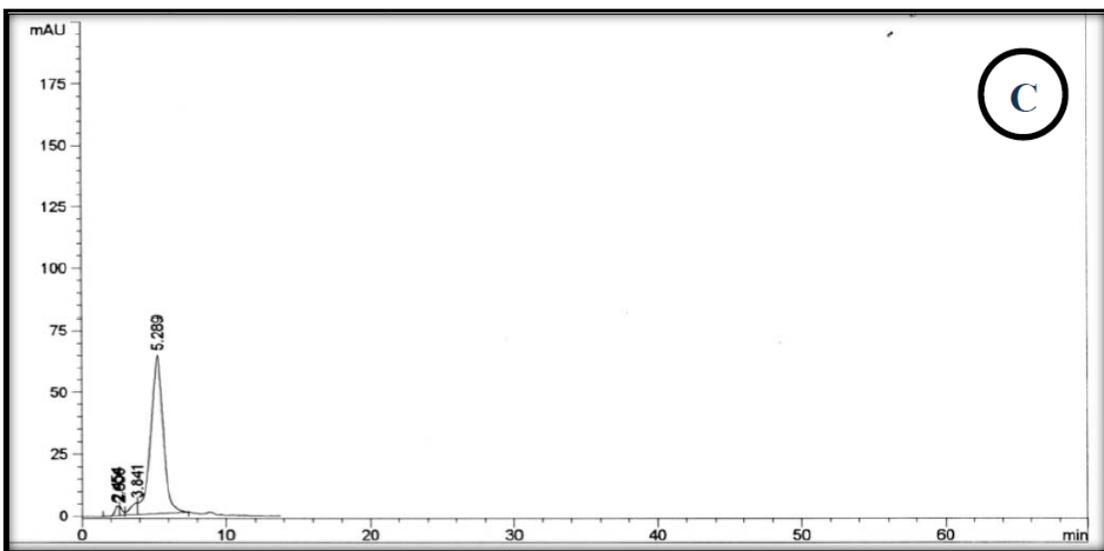
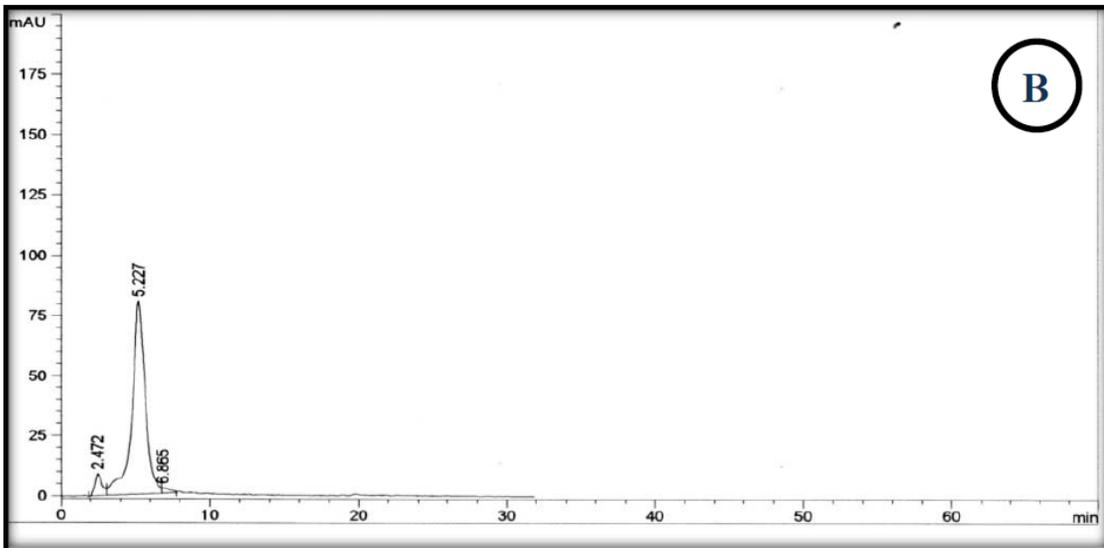
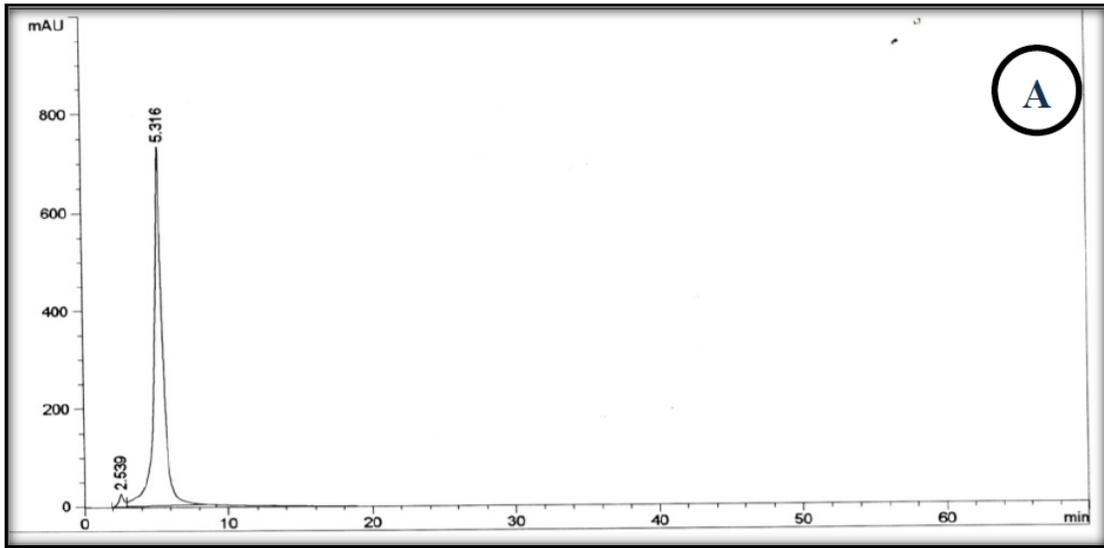


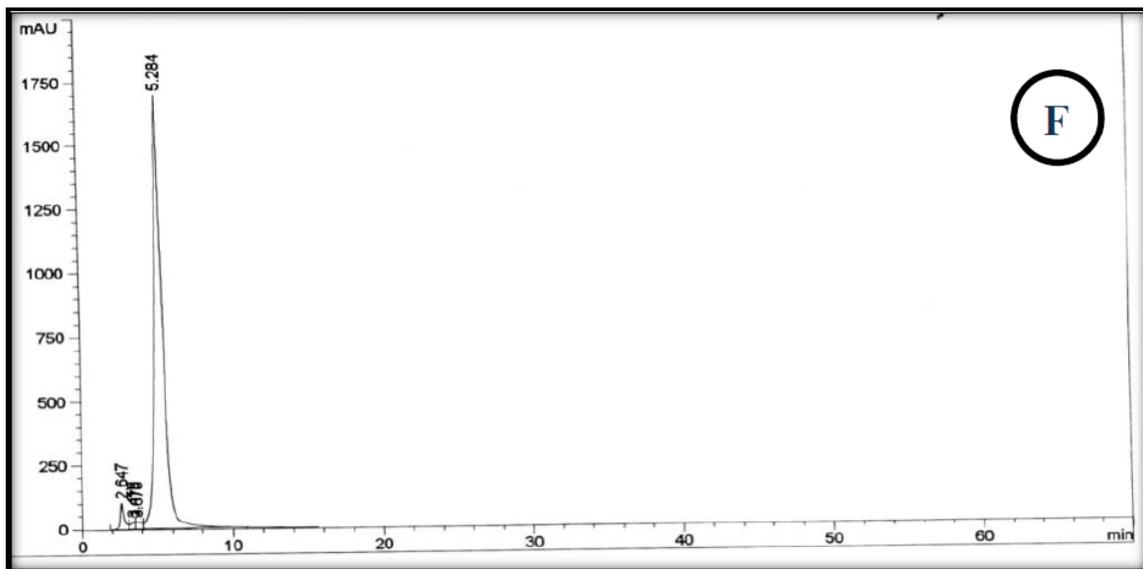
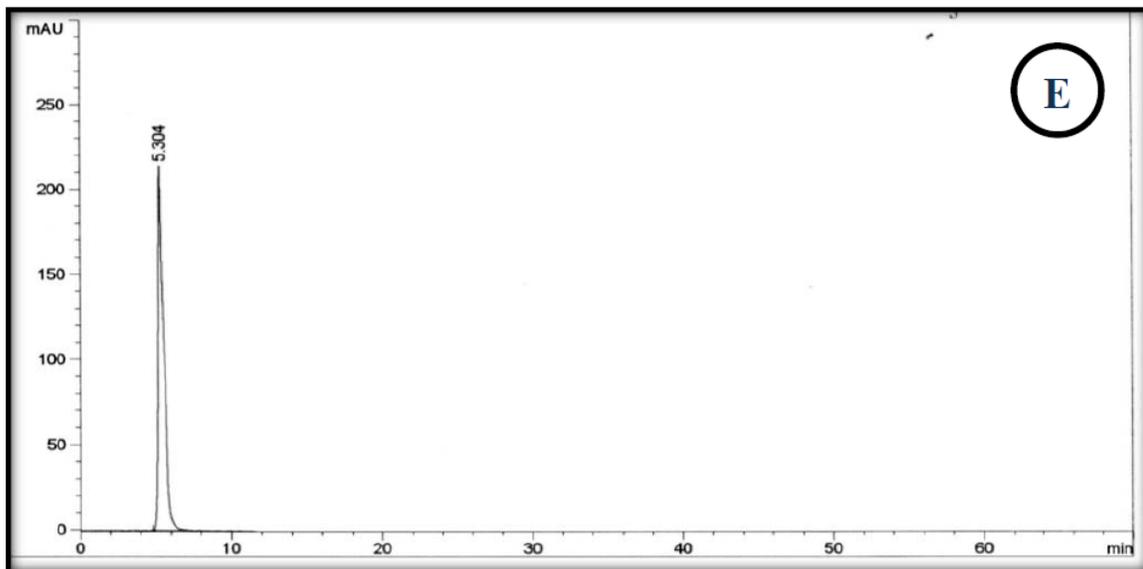
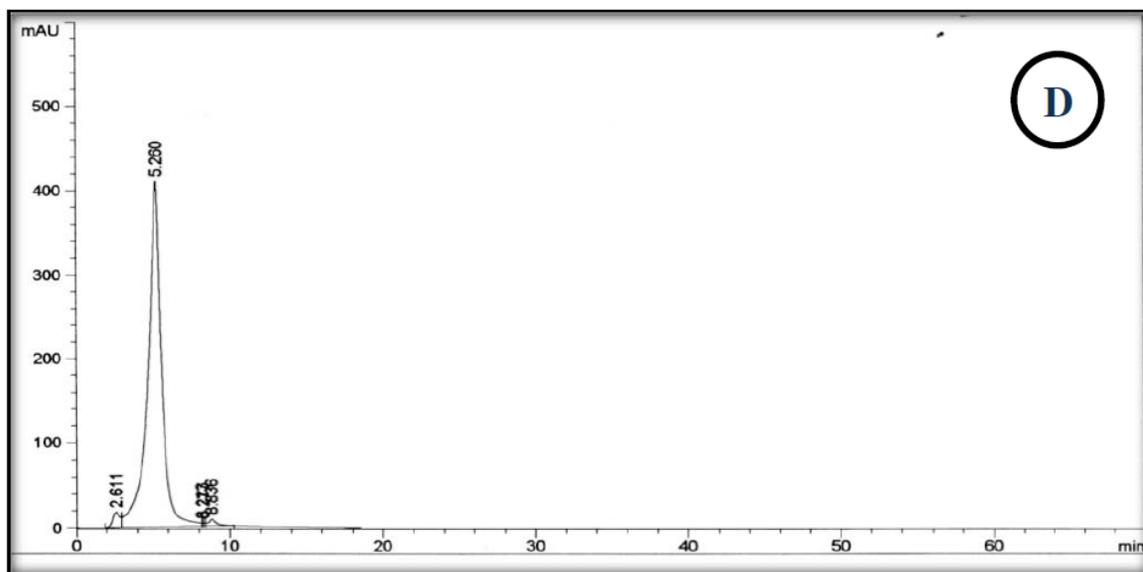
Figure 2: TLC of standard and sample solutions; **lane 1:** *in vivo* rhizome, **lane 2:** *in vitro* rhizomes, **lane 3:** *in vitro* shoots, **lane 4:** standard, **lane 5:** *in vitro* roots, **lane 6:** *in vitro* microrhizomes, **lane 7:** callus cultures and **lane 8:** standard. **A:** after sprayed with 10% sulphuric acid and **B:** after heating. Plate: Silica gel 60 F₂₅₄ (20x20 cm). Solvent system: n-hexane–acetone (8:2).

all showed similar UV spectra characteristic of 6-gingerol compounds, having a UV absorption maximum at 282 nm. Chromatograms obtained at 282nm for 6-gingerol gave the best compromise between sensitivity and baseline noise. The amounts of 6-gingerol from *in vivo* rhizomes and different ginger parts derived from *in vitro* cultures are shown in Table 2. 6-gingerol was the major ginger oleoresin. The pungency of fresh ginger is due to a series of homologous phenolic ketones, of which 6-gingerol [1-(4'-hydroxy-3'-methoxyphenyl)-5-hydroxy-3-decanone], is the most abundant. Overall results revealed that, amounts of 6-gingerol in rhizomes (*in vivo* rhizomes harvested from *in vitro* propagated plantlets and *in vitro* microrhizomes) were higher than *in vitro* calli, shoots and roots, respectively. *In vivo* rhizomes gave the highest amounts of 6-gingerol followed by rhizomes derived from *in vitro* propagated plants under greenhouse conditions (45.37

and 42.64 mg/g extract respectively); while, *in vitro* microrhizomes gave 28.11 mg/g extract. In contrast, the lowest amounts of 6-gingerol 6.40 and 7.46 mg/g extract were recorded with *in vitro* roots and shoots while the callus gave 7.89 mg/g extract. These results were in accordance with those of several authors which reported the efficiency of HPLC method that is suitable for analysis of 6-gingerol. Balladin *et al.* [20] reported that, isocratic HPLC method can be used to investigate the pungency profile of the extracted oleoresin from the ginger rhizomes. In this respect, Balladin and Headley [8] concluded that liquid chromatography method can be considered to be a cheap method for the rapid identification and quantification of the essential oils and the pungent principles (gingerol) of the extracted dried ginger rhizomes. However, Balachandran *et al.* (2006) reported that HPLC analysis showed that the ginger extract obtained from the oven-dried feed contained



(Figure 3). Continued.



(Figure 3). Continued.

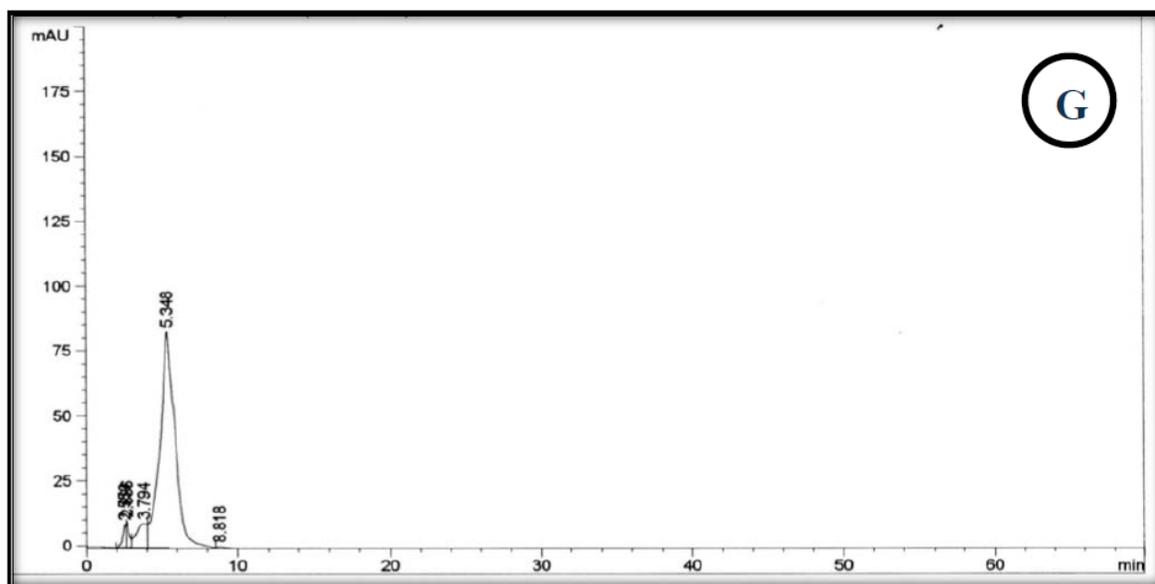


Figure 3: HPLC chromatogram showing the presence of 6-gingerol, **A:** synthetic standards, **B:** *in vivo* rhizome **C:** *in vitro* rhizomes, **D:** *in vitro* shoots, **E:** *in vitro* roots, **F:** *in vitro* microrhizomes and **G:** calli cultures.

Table 2: HPLC Quantification of 6-Gingerol in Different Parts (*In Vivo* and *In Vitro*) of *Zingiber officinale*

Extracts	6-gingerol (mg/g extract)
<i>In vivo</i> rhizome	45.37
<i>In vitro</i> Rhizomes	42.64
<i>In vitro</i> shoots	7.46
<i>In vitro</i> roots	6.40
<i>In vitro</i> microrhizomes	28.11
Calli cultures	7.89

proportionately less gingerols and more shogaols due to thermal degradation [21]. While, Jiang *et al.* (2006) reported that the *Zingiber officinale* samples showed a significant quantitative difference in non-volatile composition, particularly the content of 6-gingerols [22]. Also, Rai *et al.* (2006) developed a sensitive and accurate High-Performance Thin Layer Chromatography (HPTLC) method to determine the quantity of 6-gingerol in rhizomes of *Zingiber officinale* [23]. Methanol extracts of rhizomes from three different sources were used for HPTLC, n-hexane, and diethyl ether (40:60 v/v) as the mobile phase. The mean quantity of 6-gingerol was found to be 60.44 ± 2.53 mg/g of ginger extract. Moreover, Lee *et al.* (2007) was determined 6-, 8-, 10- gingerol, and 6-shogaol in dried extracts of ginger (*Zingiber officinale*) and the

quantification was undertaken at 200 nm [24]. The levels of 6-, 8-, 10-gingerol, and 6-shogaol in the raw herb were 9.3, 1.6, 2.3, and 2.3 mg/g, respectively. Further, Schwertner and Rios (2007) developed a HPLC method that is suitable for analysis of 6-gingerol [25]. The recoveries of 6-gingerol, from the ginger dietary supplements and ginger-containing products was 94.7. The within-day coefficients of variation for 6-gingerol, standards at 50.0 $\mu\text{g/mL}$ were 2.54. Moreover, Bailey-Shaw *et al.* (2008) studied the changes in yields of oleoresin and content of pungent bioactive principles: 6- gingerols of Jamaican ginger (*Zingiber officinale*) which analyzed by HPLC during different stages of maturity (7-9 months) and found that 6-gingerol was the most abundant component in all samples [26]. Recently, Sanwal *et al.* (2010) analyzed fresh rhizomes of 18 diploid and tetra-ploid genotypes of Indian ginger, and found that 6-gingerol was identified as the major pungent phenolic compound [27]. In addition, the total gingerol content of the tetraploid type was much higher than that of the respective diploid type.

CONCLUSION

This study sought to characterize the presence of 6-gingerol in different parts (*in vivo* and *in vitro*) of *Zingiber officinale* Rosco using thin layer chromatography (TLC) and high performance liquid

chromatography (HPLC). 6-gingerol was detected in all extracts of different parts of ginger derived from *in vivo* and *in vitro* culture conditions. TLC screening showed spots having identical R_f value (0.15), according to the synthetic standards of 6-gingerol in all samples extract. HPLC chromatogram demonstrates similar UV spectra

characteristics of 6-gingerol in synthetic standards, *in vivo* rhizomes, and *in vitro* cultures of different ginger parts. The quantity of 6-gingerol in rhizomes (*in vivo* and *in vitro*) and *in vitro* microrhizomes (45.37; 42.64; 28.11 mg/g respectively), were showed a higher value than that of *in vitro* calli, shoots and roots (7.89; 7.46; 6.40 mg/g respectively).

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