

Estimation of gingerol content in different brand samples of ginger powder and their anti-oxidant activity: A comparative study

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Abstract

Gingerol is the most abundant constituent of fresh ginger has potent antioxidant activity but it decreases during postharvest storage and processing. The present work efforts have been made to estimate *Gingerol* content in different brand samples of Ginger powder and their antioxidant activity compared with the sample cultivated through organic farming. Organic farmed sample was collected from the FRI, Dehradun (UK), other samples were taken from the markets of Srinagar Garhwal and Dehradun and was identified from the Dept. of Botany H.N.B. Garhwal University, Srinagar Garhwal (UK). All reagents and chemicals were used of analytical and HPLC grade and extraction was carried out by hot solvent extraction method. FRAP method was used for Antioxidant activity. Simple reversed-phase HPLC-UV method, with gradient elution, was used to estimate the *gingerol*. A typical HPLC-UV chromatogram was obtained which exhibited a clean and smooth baseline with excellent resolution where all the marker peaks could be identified and quantified. The amount of *gingerol* in the sample S1 is 04.54%, in S2 is 08.01%, in S3 is 06.74%, in S4 is 04.20%, in S5 is 06.74% and in S6 is 08.54%, estimated respectively by HPLC analysis. Among all samples, S6 (ginger cultivated through organic farming) has significant quantity of gingerol in comparison to other market samples. The reducing ability of different samples of ginger extracts was in the range of 368.27 ± 23.43 - $3107.28 \pm 42.31 \mu\text{mol/g}$ dry weight. The FRAP values for the methanolic extracts of rhizomes in all six varieties were significantly lower than those of vitamin C and α -tocopherol, but higher than that of BHT. When we compared all five market samples with ginger cultivated through organic farming had excellent antioxidant activity. The results conclude that ginger which cultivated through organic farming has a significant antioxidant activity and has a positive relationship between antioxidant activities and total phenolic contents. The high antioxidant activity shows the higher level of total phenolic and flavonoids.

Keywords: *Zingiber officinale*, Gingerol, HPLC-UV, HPTLC.

INTRODUCTION

Zingiber officinale also known as ginger belongs to the family *Zingiberaceae* is a slender perennial plant firstly cultivated in China and then spread to India, Southeast Asia, West Africa, and the Caribbean. The plant reaches to the height of 2 feet and has greenish yellow flowers resembling orchids with aromatic pungent taste. It is a tropical plant and its underground stem is used for culinary and medicinal purposes. The rhizome of *Zingiber officinale* is one of the most widely used species of the ginger family and is a common condiment for various foods and beverages. It is characterized in traditional Chinese medicine as spicy and hot, ginger is claimed to improve the body and tardy pulse, address a pale complexion, and strengthen the body after blood loss. In ancient China, it was regarded as a healing gift from God and was commonly used to cleanse and warm the body. It has historically been used in south eastern countries to treat many ailments including cold and flu symptoms, headaches, high blood pressure, and

hypercholesterolemia. It is also a potent antiemetic. Research studies document the effectiveness of ginger in treating nausea and vomiting postoperatively, in pregnancy and during chemotherapy [1]. Many herbalists use ginger as a remedy for motion and morning sickness, menstrual cramps and digestive disorders. Hence it is also recommended as an alternative to aspirin for people who cannot take aspirin because of its irritating effect on the gastrointestinal tract. It has a long history of medicinal use dating back 2,500 years in China and India for conditions such as rheumatism, colds, etc. [2]. It is widely used in cooking and phytotherapy because of its volatile oil and oleoresin. Nonetheless, ginger has a considerable amount of starch (up to 40%, dry basis) with potential applications, thus, the residue from SFE of ginger or the ginger bagasse can be used as a source of starch and also as substrate for hydrolysis reactions to obtain new molecules from oligosaccharides to glucose or even smaller molecules. The major active ingredients in ginger are terpenes and oleoresin called ginger oil. This active ingredient is known by the name *Gingerol* [3, 4, 5].

In the present work efforts have been made to identify and quantify the bioactive molecule i.e. *Gingerol* from different market samples (Purchased from Local area of Srinagar Garhwal and Dehradun) of *Zingiber officinale* (Rhizome) by solvent extraction method in combination with HPLC. It is also aimed to compare the anti-oxidant activity and content of their active molecule with cultivated sample.

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MATERIAL AND METHODS

Plant Material

The cultivated Ginger rhizomes were collected from the Forest Research Institute, Dehradun, (UA) and powdered samples

were taken from the market of Srinagar (Garhwal) and Dehradun with different brand names shown in [Table. 01]. All samples were identified from the Dept. of Botany H.N.B. Garhwal University, Srinagar Garhwal (UK).

Table 1. Different Samples of Ginger and Their Brand Names

S.No.	Sample Codes	Brand Names
01.	S1	MVJ Foods (India) Pvt. Ltd. Cochin (The Spices City of India)
02.	S2	Jai Ram Dass Khushiram, Maharashtra, India
03.	S3	Herbal Bio-Solutions, Delhi, India.
04.	S4	Green Earth Products Pvt. Ltd., Delhi.
05.	S5	Mother Herbs Pvt Ltd., Padpadganj, Delhi.
06.	S6-Cultivated (Organic farming)	FRI, Dehradun, India.

Chemicals and Regents

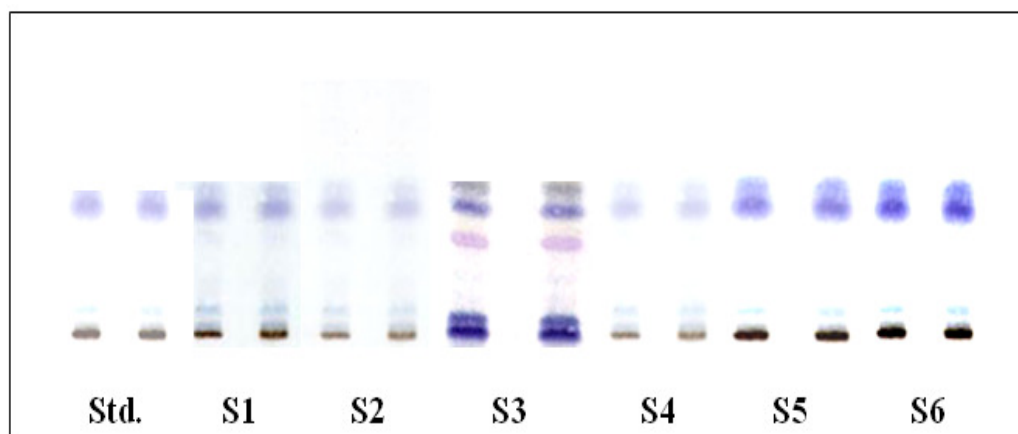
All reagents and chemicals were used of analytical and HPLC grade.

Sample preparation : The mark from petroleum ether fraction of *Zingiber officinale* rhizome powder extract was successively extracted with methanol. The extracts were concentrated and separately applied on different TLC plates.

Identification of Gingerol by TLC

Solvent front run upto : 9 cms
Application : CAMAG Linomat IV
Detection : Vanillin sulphuric acid [Fig. 1]
Scanning : 254 nm [Fig. 2a and 2b]

Sample detail : *Zingiber officinale* rhizome powder.
Adsorbent : Precoated silicagel (Al - Sheet)
Mobile Phase : n-Hexane: Ether 40: 60



S2: Sample-2, S3: Sample-3, S4: Sample-4, S5: Sample-5, S6: Cultivated Sample.

Fig 1. Identification of Gingerol from rhizome powder of *Zingiber Officinale* by TLC.

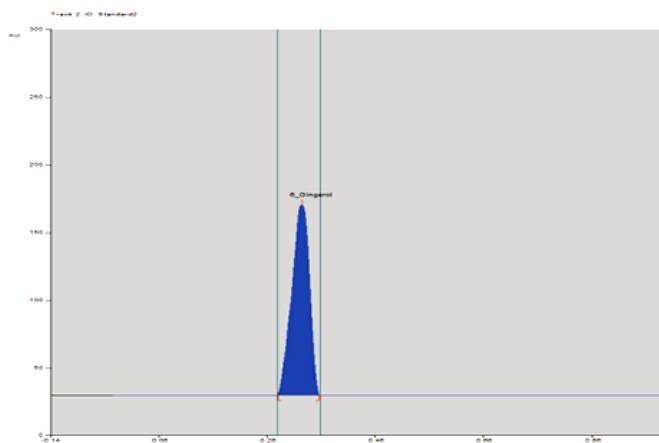


Fig 2a. HPTLC Chromatogram of standard Gingerol.

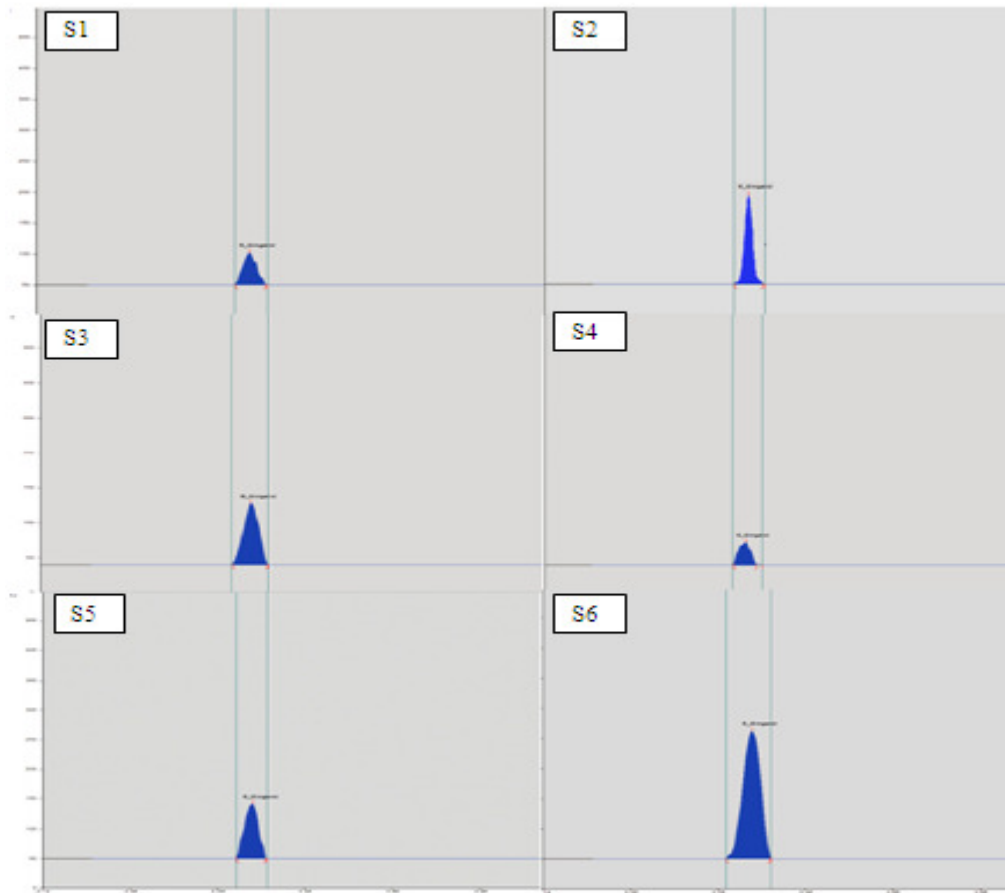


Fig 2b. HPTLC Chromatogram of S1, S2, S3, S4, S5 and S6 Samples.

Estimation of Gingerol Content in *Zingiber officinale* Rhizome Extracts by HPLC Extraction Process

5 g of the coarsely powdered substances under examination 100 ml methanol on water bath for 15 min cool and filter. Reflux the residue further with methanol till the test extract turns colourless, cool and filter. Combine all the filtrate and concentration to 50 ml.

Analysis [7]

Chromatographic system: High Performance Liquid Chromatographic system equipped with LC8A pump, SPD-M 10Avp Photo Array Detector in combination with Class LC 10A software.

Mobile phase: Acetonitrile: Water 55 : 45

Column: C18 - ODS (Octadecylsilane) (Lichrocart 250-4, Lichrospher RP-18e-5m (Merck) Art.No: 1.50216

Detector: SPD-M 10Avp Photo diode array detector

Flow rate: 1.3 ml/min

Wave length: 280 nm

Injection volume: 10 μ L

Standard preparation: Weigh accurately 100mg of working standard (contains 40% w/w of Gingerol) to a 25ml volumetric flask. Dissolve and make upto 25ml with HPLC grade methanol.

Sample preparation: Weigh accurately a sample quantity equivalent to 40 mg of gingerol to a 25 ml volumetric flask. Dissolve and make upto 25ml with HPLC grade methanol.

Procedure: Set the instrument as per the chromatographic condition prescribed above. By means of suitable syringe inject 10 μ l of standard solution. Record the chromatograms repeat the injections for another 4 times and calculate the RSD of the area. It should not be more than 2%. Inject 10 μ L of sample preparation and record the chromatogram. Calculate the % of gingerol from the peak areas.

Total antioxidant activity (FRAP assay):

A slightly modified method of was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer (3.1 g CH₃COONa and 16 ml CH₃OOH, pH 3.6), 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl₃·6H₂O solution. This assay involved (i) preparation of fresh FRAP solution by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml FeCl₃·6H₂O, (ii) raising temperature of the solution to 37°C, (iii) allowing plant extracts (150 μ L) allowed to react with 2850 μ l of the FRAP solution for 30 min in the dark and (iv) taking readings of the coloured product (ferrous tripyridyl tria-zine complex) at 593 nm. The standard curve was linear between 200 and 1000 μ M FeSO₄. Results are expressed

in $\mu\text{M Fe (II)}/\text{g dry mass}$ [8].

RESULT AND DISCUSSION

Simple reversed-phase HPLC-UV method, with gradient elution, was used to qualitatively and quantitatively, estimation of the *gingerol* in plant extracts of *Z. officinale*. A typical HPLC-UV chromatogram was obtained using gradient elution of a crude extract, which exhibited a clean and smooth baseline with excellent resolution where all the marker peaks could be identified and quantified. The Gingerol show a characteristic UV absorption maximum at 280nm. In addition to UV absorption, retention times (Rt) in reversed-phase (RP)-HPLC were also found to be useful in the structure confirmation and especially for compounds belonging to homologous series.

The amount of *gingerol* in the sample S1 is 04.54%, in S2 is 08.01%, in S3 is 06.74%, in S4 is 04.20%, in S5 is 06.74% and in S6 is 08.54%, estimated respectively by HPLC analysis [Table .2.] [Fig: 3-10]. In the rhizomes, the antioxidant potentials in five different

market varieties and a cultivated sample of *Zingiber officinale* were estimated from their ability to reduce 2,4,6-tripyridyl-s-triazine (TPTZ)-Fe (III) complex to TPTZ-Fe (II) [Table 03]. Maximum antioxidant activity was found to be 767.2 $\mu\text{mol/g dry weights}$ in the S6 sample whereas least activity was found in the S4 sample (368.27 $\mu\text{mol/g}$). The FRAP values of all six varieties were significantly lower than those of vitamin C and α -tocopherol, but higher than that of BHT. When we compared all five market samples with ginger cultivated through organic farming had excellent antioxidant activity. Besides these, sample S1, S2, S3 and S5 has 376.94, 680.68, 0579.6 and 0537.94 $\mu\text{mol/g dry weights}$ ferric reducing ability respectively.

Again when we compare the reducing ability and amount of Gingerol among the brand samples then, the Jai Ram Dass Khushiram, Maharashtra, India (S2) brand has a significant antioxidant activity (680.68 $\mu\text{mol/g}$) and Gingerol content (08.01%) which is more near about the value of cultivated sample (S6-767.2 $\mu\text{mol/g}$) whereas Green Earth Products Pvt. Ltd., Delhi (S4) brand has least activity (368.27 $\mu\text{mol/g}$) and Gingerol content (04.20%).

Table 2. Amount of gingerol in different plant samples of *Z. officinale* analyzed by HPLC.

Sample Detail (From Dehradun Market)	Estimation of gingerol (%) using HPLC
S1	04.54
S2	08.01
S3	06.74
S4	04.20
S5	06.74
S6	08.54

Table 3. Total antioxidant (FRAP) activity in different rhizome samples of *Zingiber Officinale*. BHT, α -tocopherol and Vitamin C were used as positive controls. All analyses were the mean of triplicate measurements \pm standard deviation.

S.N.	Sample Detail (From Dehradun Market)	Estimation of antioxidant activity by FRAP method ($\mu\text{mol/g dry weight}$)	
01.	S1	376.94 \pm 50.97	
02.	S2	680.68 \pm 18.38	
03.	S3	0579.6 \pm 61.94	
04.	S4	368.27 \pm 23.43	
05.	S5	0537.94 \pm 37.3	
06.	S6	0767.2 \pm 41.53	
07.	Standards	BHT	074.31 \pm 11.21
		α -tocopherol	0953.0 \pm 23.41
		Vitamin C	3107.28 \pm 42.31

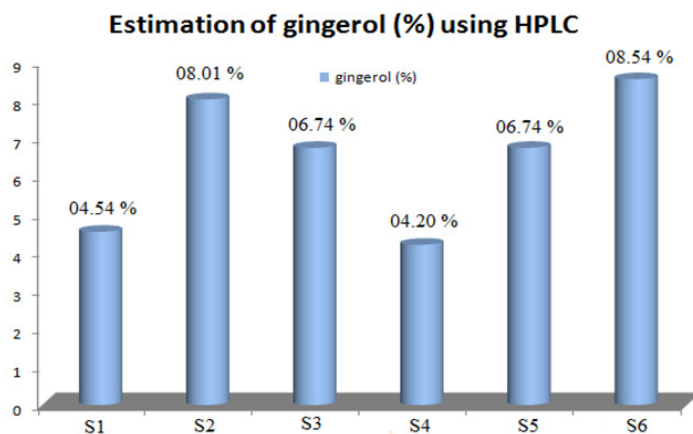


Fig 3. Estimation of Gingerol Content in Different Ginger Samples Using HPLC

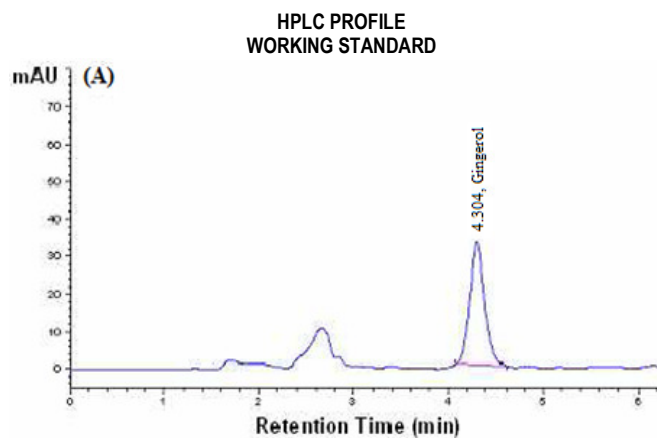


Fig 4. HPLC Chromatogram of standard Gingerol.

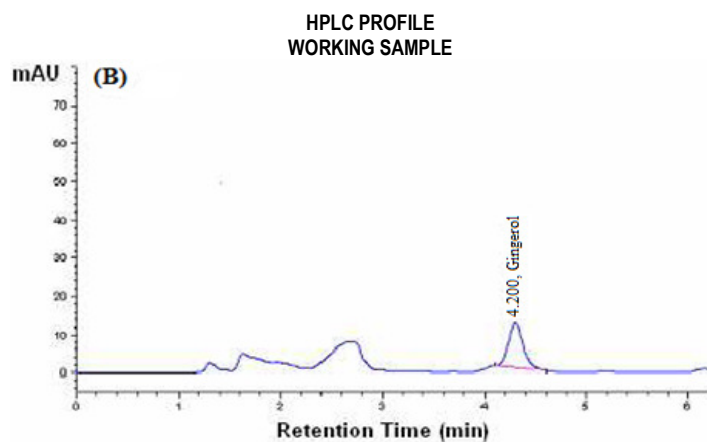


Fig 5. HPLC Chromatogram of S1.

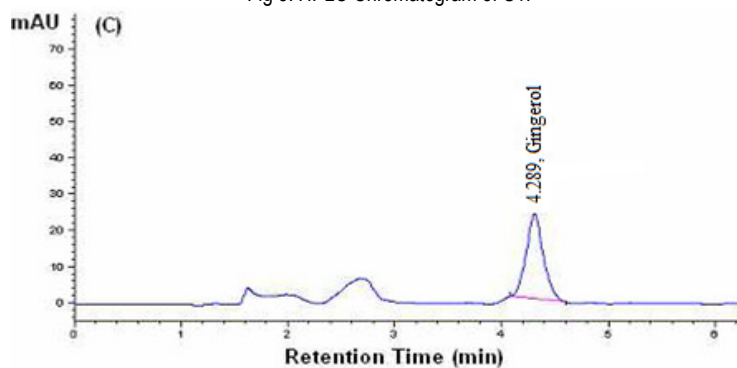


Fig 6. HPLC Chromatogram of S2.

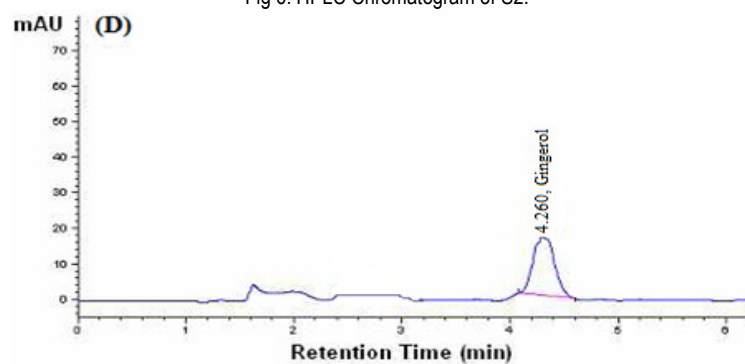


Fig 7. HPLC Chromatogram of S3.

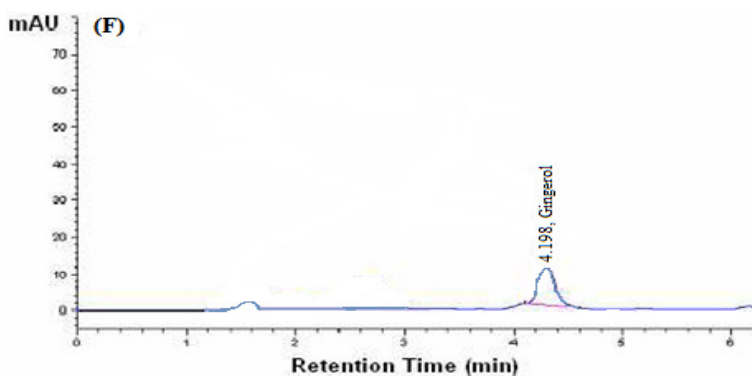


Fig 8. HPLC Chromatogram of S4.

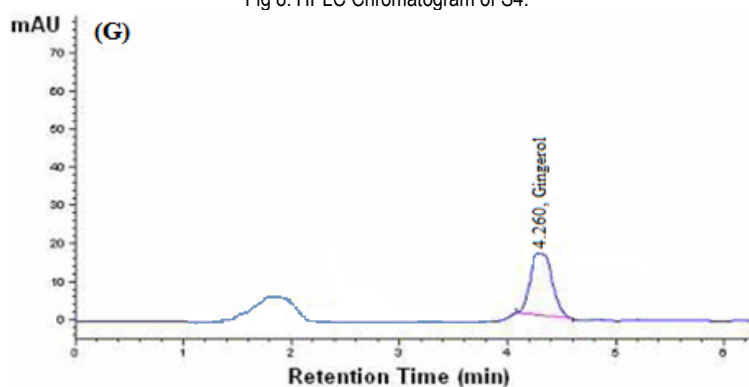


Fig 9. HPLC Chromatogram of S5.

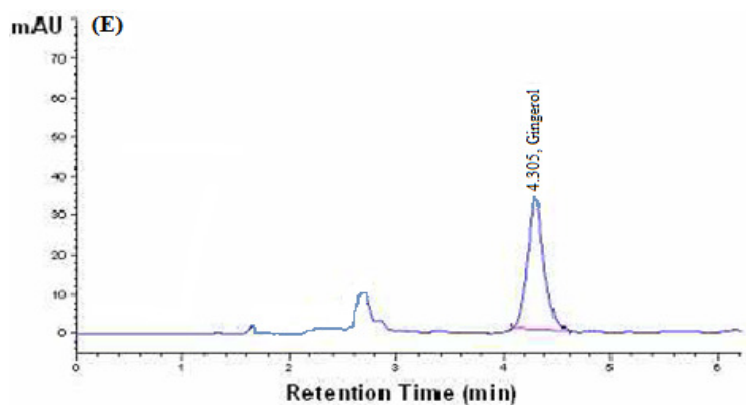


Fig 10. HPLC Chromatogram of S6.

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