



# Qualitative and Quantitative Estimation with Isolation of Gingerol From *Zingiber Officinale* Rhizome

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## KEYWORDS

*Zingiber officinale*,  
Gingerol, TLC,  
Chromatography

## ABSTRACT:

The study aims to comprehensively analyze the bioactive compounds present in the rhizome of *Zingiber officinale*, commonly known as ginger, with a particular focus on *gingerol*, the principal bioactive component. This research encompasses both qualitative and quantitative assessments to identify and measure the concentration of *gingerol*. The qualitative analysis involves the preliminary phytochemical screening of the ginger rhizome extract to detect the presence of various secondary metabolites, including alkaloids, flavonoids, terpenoids, and phenolic compounds. The methanolic extract showed a total phenolic content of 187.10 mg/gm and a total flavonoids content of 171.33 mg/gm, as determined by gallic acid equivalents and Rutin equivalents. Techniques such as Thin Layer Chromatography (TLC) and UV spectroscopy are employed to confirm the presence of *gingerol* and other related compounds. The extraction process utilizes solvent extraction methods, optimizing parameters such as solvent type, extraction time, and temperature to maximize yield. After performing extraction of *Zingiber officinale*, the percentage yield of Rhizome extract in different solvents like petroleum ether and methanolic extract were found to be 0.91 % (0.915 gm) and 6.08 % (6.028 gm) respectively. Isolation of *gingerol* is achieved through a combination of chromatographic separation techniques. Initially, the crude extract is subjected to column chromatography to fractionate the components, followed by UV spectroscopy. The isolated *gingerol* is then characterized using spectroscopic methods like Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS) to confirm its structure and purity. The findings of this study provide a detailed understanding of the chemical composition of ginger rhizome, with precise quantification and successful isolation of *gingerol*. The study not only establishes a methodological framework for the extraction and analysis of bioactive compounds from medicinal plants but also highlights the potential of *Zingiber officinale* as a rich source of health-promoting constituents.

## 1. Introduction

In the production of pharmaceuticals, whether for synthetic, pharmacopoeial, or non-pharmacopoeial uses, medicinal herbs and plants are regarded as essential elements that can also be utilized in food preparation. According to Sandberg and Corrigan (2001), plants are an essential source of medicine and have a major impact on human health. As secondary metabolites, phytochemicals are the bioactive substances derived

from plants, even though the plants that produce them may not require those (Molyneux *et al.*, 2007). Every part of the plant, including the leaves, stem, bark, roots, flowers, fruits, seeds, and so on, can produce them and contain active components. Together with fibers and minerals, these substances create an integrated component of the immune system that protects against a variety of illnesses and stressful situations (Edeoga, 2005). The therapeutic and medical benefits of aromatic and medicinal plants have been recognized since



ancient times. Using natural molecules instead of synthetic ones has been the subject of numerous scientific studies in recent years. This is primarily because plant material contains a wide variety of molecules with various applications in the food, pharmaceutical, and environmental industries (Bahorun *et al.*, 1996). Everybody should use medicinal plants in their regular diets since they are excellent ingredients. Natural remedies for a wide range of ailments have been utilized historically and in folklore from prehistoric times. Worldwide, people utilize ginger (*Zingiber officinale*) as a spice, condiment, and herbal medicine in their cookery. In the past, this ubiquitous spice has been used to cure a number of illnesses (Mbaveng and Kuete, 2017). *Zingiber officinale*, generally known as ginger, is a flowering plant that is native to India, China, Southeast Asia, the West Indies, Mexico, and several other parts of the world. 1. Among the tastiest and healthiest foods is ginger. The underground portion of the stem, known as the rhizome, is frequently used as a spice. It's one of the most often used herbs in traditional medicine, aside from being used as a spice. It is closely related to galangal, cardamom, and turmeric. It is a member of the Zingiberaceae family. Worldwide, ginger is used as a spice and flavoring, and it has many known health benefits, such as pharmacological effects, antioxidant, antibacterial, anti-inflammatory, antinociceptive, anti-mutagenic, and hepatoprotective properties (Wan-Nadilah *et al.*, 2019; Munda *et al.*, 2018; Mahboubi, 2019). The rhizomes of *Zingiber officinale* Roscoe, a member of the Zingiberaceae family, are known as ginger and are among the most well-known spices in the world. It has been used for over 2,000 years and is a key plant in Greek, Ayurvedic, and Chinese medicine (Awe *et al.*, 2013). Furthermore, ginger was a component of the mummification methods used in ancient Egypt (Gigon, 2012). The plant's various chemical constituents are what give it its therapeutic benefits and pharmacological uses, including anti-inflammatory, anti-diabetic, antibacterial, antifungal, anti-oxidant, anti-tumor, anti-cancer, anti-proliferative, and anti-platelet effects (Mbaveng and Kuete, 2017; Guk Shin *et al.*, 2005). The goal of the current investigation was to assess the Bioactive content of ginger powder by extraction and isolation. The phenols, flavonols, and flavonoides found in ginger extracts. We performed TLC, Column chromatography, UV spectroscopy, FT-

IR spectroscopy, Mass spectroscopy and  $^1\text{H}$  NMR analysis to determine the chemical composition of the bioactive component of methanol extract of ginger rhizomes.

## 2. Material and Method

### 2.1 Plant material

The medicinal plant *Zingiber officinale* rhizome was collected from local market of Bhopal. Authentication of selected traditional plant - Medicinal plant *Zingiber officinale* rhizome was authenticated by a plant taxonomist in order to confirm its identity and purity. The **Authentication No.2021077** was provided for the medicinal plant.

### 2.2 Soxhlet extraction:

The dried and powdered *Zingiber officinale* rhizome is first defatted with petroleum ether and then added to a Soxhlet apparatus thimble. Using a methanol solvent solution, the extraction was done for eight to ten hours at the heating mantle's temperature of 40 to 60 degrees Celsius. Following the extraction procedure, the sample extract was filtered and dried out. The extracted materials were evaporated at 40°C in a rotary vacuum evaporator. Extracts were gathered and kept in an airtight container (Alara *et al.*, 2019). Extraction yield of all extracts were calculated using the following equation below:

$$\text{Formula of Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

### 2.3 Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in methanol extract of *Zingiber officinale* using standard procedures (Kokate *et al.*, 2006).



## 2.4 Quantitative Phytochemical estimation-

### 2.4.1 Spectrophotometric Quantification of Total Phenolic Content: -

The total phenolic content of the plant extract was measured using the Folin-Ciocalteu Assay. The *Zingiber officinale* extract (0.2 mL from the stock solution) was combined with 2.5 mL of Folin-Ciocalteu's phenol reagent. After 5 minutes, 2 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution was added to the mixture. This combination was diluted with distilled water to a maximum of 7 mL. The mixture was left in the dark for 90 minutes at 23°C before the absorbance was measured at 760 nm. The TPC was calculated by extrapolating a calibration curve created by making gallic acid solution (20 to 100 µg/mL). The phenolic chemicals were estimated in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram dried material (TanZoo *et al.*, 2015).

### 2.4.2 Spectrophotometric Quantification of Total Flavonoids Content: -

The flavonoids content was determined using Aluminium chloride method (Chang *et al.*, 2002). In a 10 mL test tube, 0.5 mL of *Zingiber officinale* extract, 0.15 mL of NaNO<sub>2</sub> (5 %) and 0.15 mL of AlCl<sub>3</sub>.6H<sub>2</sub>O (10 %) was mixed. After 5 min, 2 mL of NaOH (4 %) was added. The solution was mixed well and the absorbance was measured at 510 nm. The standard curve for total flavonoids was made using rutin standard solution (20 to 100 µg/mL) under the same procedure as earlier described. The total flavonoids were expressed as milligrams of rutin equivalents per g of dried fraction (Parthasarathy *et al.*, 2009).

## 2.5 Preliminary Thin layer chromatography:-

Using several solvent systems, *Zingiber officinale* extract was thin-layered chromatographed using silica gel 60 F254 pre-coated TLC plates with a 0.2 mm layer thickness. A capillary tube was used to manually apply the spots, an air blower was used to dry the plates, and a room-temperature solvent system was used to develop the TLC chamber. Spots on TLC plates were observed under UV light after being sprayed with a sulfuric acid solution reagent. R<sub>f</sub> values were calculated (Kumar *et al.*, 2018).

$$R_f \text{ Value} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Solvent system developed in preliminary TLC for ZO methanolic extract in which the maximum spots were visible in n-hexane: diethyl ether (4:6) mobile phase with std. Phenolic. So that n-hexane: diethyl ether (4:6) solvent was taken as mobile phase for column chromatography.

## 2.6 Column chromatography

Silicagel column chromatography was used to separate the phenolic from the *Zingiber officinale* extract in methanolic extract. Chromatography was performed using a vertical glass column composed of borosilicate material. Before packing, the column was thoroughly dried and cleaned with acetone. Wet packing was used to pack the column, with silica gel (60–120) serving as the adsorbent. Toluene was used to produce the slurry, which was then added to the column. One gram of extract was applied to the column's top. Column chromatography was performed using the gradient elution technique. N-hexane:diethyl ether (4:6) was used to elute the column, and a number of elutes were collected. To find a single compound, TLC was used to concentrate the fractions or elutes that had been collected (Srivastava *et al.*, 2021).

## 2.7 Spectroscopic characterization:-

### 2.7.1 UV-visible Spectroscopy

The isolated fraction (I) of ZO Extract was scanned from 200 to 800 nm wavelength using UV-Visible Spectrophotometer (Shimadzu UV-1700) and the characteristic peaks were detected and recorded (Patel *et al.*, 2022).

### 2.7.2 FT-IR

To establish the presence of the functional groups in the isolated fraction (I) of ZO Extract, FT-IR spectroscopy was performed using Perkin Spectrum BX spectrophotometer. The sample was dried and ground with KBr pellets and analyzed on Thermo Nicolet model 6700 spectrum instrument. A disk of 200 mg of KBr was prepared with a mixture of 2% finely dried sample and then examined under IR-spectrometer. Infrared spectra were recorded in the region of 400 - 4,000 cm<sup>-1</sup> (Luciene *et al.*, 2008).



### 2.7.3 NMR Spectroscopy

NMR spectroscopy was performed for the isolated fraction (I) of ZO Extract to identify the structure of the compound present in the isolated fraction. JEOL RESONANCE NMR spectroscopy for this purpose was Fourier Transform Nuclear Magnetic Resonance spectroscopy (Zia *et al.*, 2019).

### 2.7.4 Mass Spectroscopy

Mass spectrometry converts molecules into ions and according to their mass and charge the ions can be separated and sorted. The mass spectrometer used for the identification of the molecular weight of isolated fraction (I) of ZO Extract was recorded on mass spectrometer instrument microTOF-MS. (Wiley *et al.*, 1995).

## 3. Results

### 3.1 Plant Collection

**Table 1:** Plant collection

S. No.	Plant name	Plant part used	Weight
1.	<i>Zingiber officinale</i>	Rhizome	100 gm

### 3.2 Percentage yield

**Table 2:** Percentage yield of extracts

S. No.	Plant name	Solvent	Color of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	<i>Zingiber officinale</i>	Petroleum Ether	Yellow to Brownish	100	0.915	0.91
2.	<i>Zingiber officinale</i>	Methanol	Dark Brown	99.00	6.028	6.08

### 3.3 Solubility determination of Hydro alcoholic extract of *Zingiber officinale* -

**Table 3:** Solubility Determination of Hydro alcoholic extract of *Zingiber officinale*

S. No.	Solvent	Result
1.	Water	Sparingly Soluble
2.	Ethanol	Soluble
3.	Ethyl Acetate	Sparingly Soluble
4.	DMSO	Soluble
5.	Petroleum Ether	Partially Soluble
6.	Methanol	Soluble
7.	Chloroform	Soluble
8.	Acetone	Partially Soluble



### 3.4 Qualitative Phytochemical Analysis of Hydro alcoholic extract of *Zingiber officinale*

**Table 4 :** Phytochemical analysis of methanolic extract of *Zingiber officinale*

S. No.	Experiment	Result	
		Petroleum ether	Methanolic
Test for Carbohydrates			
1.	Molisch’s Test	+	+
2.	Fehling’s Test	-	+
3.	Benedict’s Test	+	+
4.	Bareford’s Test	-	+
Test for Alkaloids			
1.	Mayer’s Test	+	+
2.	Hager’s Test	-	+
3.	Wagner’s Test	-	-
Test for Terpenoids			
1.	Salkowski Test	+	+
2.	Libermann-Burchard’s Test	-	+
Test for Flavonoids			
1.	Lead Acetate Test	-	+
2.	Alkaline Reagent Test	-	+
Test for Tannins and Phenolic Compounds			
1.	FeCl <sub>3</sub> Test	+	+
2.	Lead Acetate Test	-	+
3.	Gelatine Test	-	+
Test for Saponins			
1.	Froth Test	+	+
Test for Protein and Amino acids			
1.	Ninhydrin Test	-	-
2.	Biuret’s Test	-	+
Test for Glycosides			
1.	Legal’s Test	-	+
2.	Keller Killani Test	-	+
3.	Borntrager’s Test	-	+



### 3.5 Quantitative Phytochemical analysis *Zingiber officinale* extract

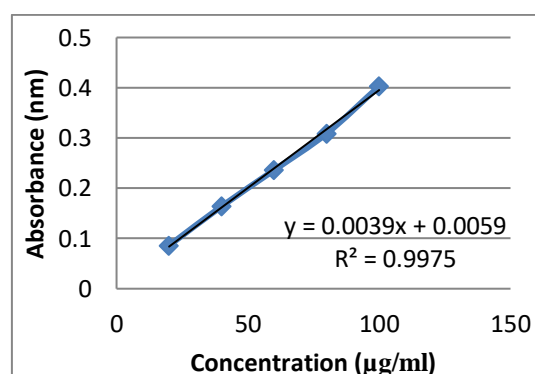
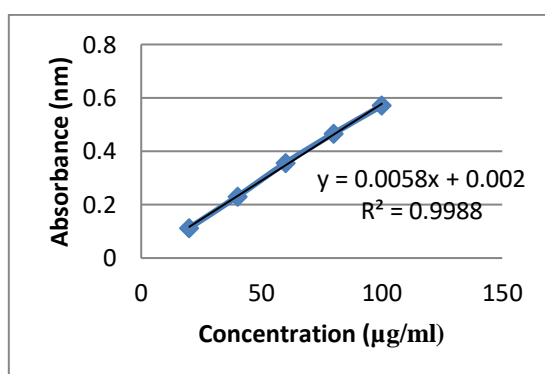
#### 3.5.1 Total Phenolic Content (TPC) & Total Flavonoid Content (TFC) Estimation

**Table 5** Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.114
2.	40	0.234
3.	60	0.353
4.	80	0.464
5.	100	0.570

**Table 6** Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.084
2.	40	0.162
3.	60	0.234
4.	80	0.307
5.	100	0.401



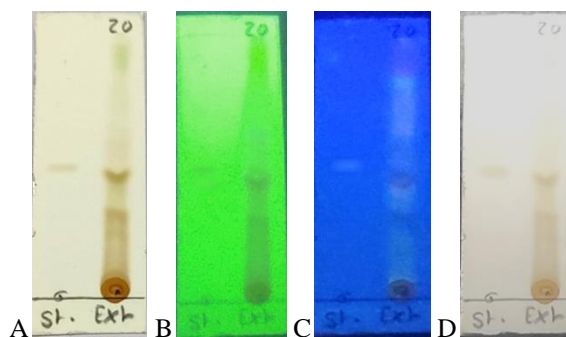
**Graph 1** Graph represent standard curve of Gallic acid (A) and Rutin (B)

**Table 7** Total Phenolic Content in *Zingiber officinale* extract

Total Phenolic content (mg/gm equivalent to Gallic acid)	
Extracts	<i>Zingiber officinale</i> extract
Absorbance Mean±SD	0.9375±0.004
TPC	187.10

**Table 6** Total Flavonoid Content in *Zingiber officinale* extract

Total Flavonoid content (mg/gm equivalent to Rutin)	
Extracts	<i>Zingiber officinale</i> extract
Absorbance Mean $\pm$ SD	0.5190 $\pm$ 0.002
TPC	171.33

**3.6 Preliminary TLC preparation for the estimation of active constituents –****TLC of *Zingiber officinale* methanolic extract****For Phenolic. :-****Mobile Phase-** n-hexane: diethyl ether (2:3)**Figure 1** TLC of ZO (A) & TLC estimation by UV lamp for ZO with Std. Phenolic (Short-UV (254nm) (B), Visible (365nm) (C), Light)**Table 7** TLC of *Zingiber officinale* Methanolic extract

S. No.	Solvent system	No. of spots	Colour of spots at Wavelength (365nm)	Colour of spots at Wavelength (254 nm)	Rf value (Extract)	Rf value (Std. Phenolic)
1.	n-hexane: diethyl ether (2:3)	10	Florescence (Std.)	Dark Green (Std)	-	0.48
			Florescence	Green	0.24	
			Blue	Dark Green	0.30	
			Florescence	Green	0.34	
			Blue	Dark Green	0.40	
			Florescence	Light Green	0.48	
			Light yellow	Light Green	0.59	
			Florescence	Green	0.76	
			Pink	Green	0.84	





			Purple	Green	0.91	
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TLC of ZO extract was performed on different solvent systems (solvent system was selected on the basis of literature survey). TLC performed in n-hexane: diethyl ether (4:6) that were clearly visible bands of ZO Extract with Std. Phenolic. The R<sub>f</sub> values of ZO Extract with Std. Phenolic. were found to be 0.48 and 0.48.

### 3.7 Column Chromatography

The fractions/elutes obtained from silica gel column chromatography of *Zingiber officinale* methanolic extract was tested for the detection of various phyto compounds using TLC. The collected fractions/elutes were taken properly and do the UV spectrum.

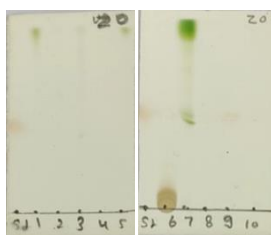
#### 3.7.1 Column Chromatography of ZO Methanolic extract –

**Table 8** Fraction collected from Column Chromatography of ZO Methanolic extract

Sr. No.	Eluent composition	Fraction collected	Remarks
1	n-hexane: diethyl ether (4:6)	01 (A)	Greenish coloured mixture of compound
2		02 (B)	White coloured mixture of compound
3		03 (C)	Light Yellowish coloured mixture of compound
4		04 (D)	White coloured mixture of compound
5		05 (E)	Creamy coloured mixture of compound
6		06 (F)	Yellowish coloured mixture of compound
7		07 (G)	Greenish coloured mixture of compound
8		08 (H)	White creamy coloured mixture of compound
9		09 (I)	Light Yellowish coloured mixture of compound
10		10 (J)	White creamy coloured mixture of compound

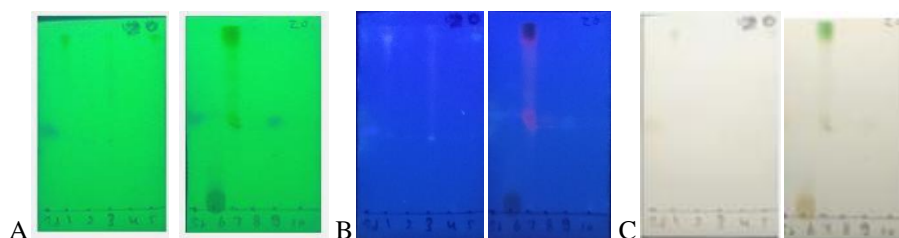
#### 3.7.2 TLC of all collected fractions-

##### A) TLC of all collected fractions of ZO Methanolic extract -



**Figure 2** TLC estimation for ZO fractions after column chromatography with Std. Phenolic.





(a) Short-UV (254 nm), (b) Long-UV (365 nm), (c) Visible Light

**Figure 3** TLC estimation by UV lamp for ZO fractions after column chromatography with Std. Phenolic. a) Short-UV (254 nm), b) Long-UV (365 nm), c) visible light.

(Std.= Standard, ZO = *Garcinia Cambogia*)

**B) TLC of fractions (A, B, C, D, E, F, G, H, I & J) of ZO Methanolic extract -**

**Table 9** Rf values of all collected fractions of ZO after column chromatography

Sr. No.	Fraction	Solvent system	No. of spots	Colour of spots at Wavelength (365 nm)	Rf value (Extract)	Rf value (Std. Phenolic)
1.	A	n-hexane: diethyl ether (4:6)	02	Fluorescence (365) Purple	0.94 0.97	0.49
2.	B		-	-	-	
3.	C		02	Fluorescence Fluorescence	0.42 0.98	
4.	D		-	-	-	
5	E		01	Fluorescence	0.86	
6	F		01	Fluorescence	0.54	
7	G		03	Pink Pink Brown	0.52 0.89 0.97	
8	H		-	-	-	
9	I		01	Fluorescence	0.48	
10	J		-	-	-	

Rf value Resulted after performing the TLC estimation was also done for the confirmation of active constituent in fraction (I) of ZO methanolic extract with mobile phase n-hexane: diethyl ether (2:3) by comparing with Std. Phenolic.

### 3.8 Spectroscopic characterization:-

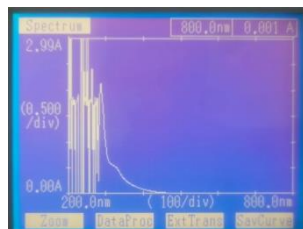
#### 3.8.1 Active constituents estimation By UV-Spectroscopy-

UV-Spectra of isolated fraction (I) of ZO Methanolic extract was recorded with a Shimadzu 1700 double beam-UV-VIS spectrophotometer. A UV spectrum of



the isolated fraction was recorded in solvent as n-hexane: diethyl ether (2:3) over a scanning range of 200-800 nm and  $\lambda_{\text{max}}$  of isolated compound were

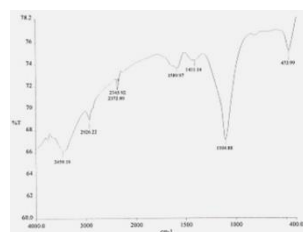
determined. The Blank was n-hexane: diethyl ether (2:3). The wavelength of isolated fraction (I) of ZO methanolic extract was found to be 284 nm.



**Figure 4** Active constitutes estimation By UV- Spectra of (A) fraction of ZO Methanolic extract after column chromatography

### 3.8.2 Active constitutes estimation By FTIR – Spectroscopy

#### (A) IR spectra of the isolated fraction (I) of ZO Methanolic extract



**Figure 5** IR spectra of the isolated fraction (I) of ZO Methanolic extract

**Table 10** FTIR- Spectrum Frequency Range of the isolated fraction (I) of ZO Methanolic extract

Sr. No.	Fraction	Frequency Range (cm <sup>-1</sup> )	Group Absorption (cm <sup>-1</sup> )	Appearance	Group	Compound Class
1	I	3550-3200 (cm <sup>-1</sup> )	3459.19	Strong, Broad	O-H stretching	Hydroxyl Group
		3000-2840 (cm <sup>-1</sup> )	2926.22	Medium	C-H stretching	Alkane
		2400- 2000 (cm <sup>-1</sup> )	2372.80	Strong	C-H stretching	Alkane
		1600–1578 (cm <sup>-1</sup> )	1589.97	Medium	C-O stretching	Carbonyl group
		1420-1330 (cm <sup>-1</sup> )	1411.14	Medium	O-H bending	Alcohol
		1400- 1100 (cm <sup>-1</sup> )	1104.88	Weak	C-C stretching	Alkane



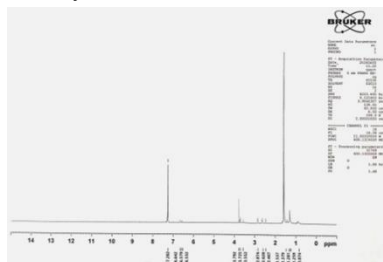
The IR Spectra of isolated fraction (I) of ZO Methanolic extract showed that -OH group Strong, Broad peak appeared at  $3459.19\text{ cm}^{-1}$ , C-H stretching peaks of Alkane at  $2926.22$  &  $2372.80\text{ cm}^{-1}$ . The Carbonyl group C=O stretching peak at  $1589.97\text{ cm}^{-1}$ , O-H bending peak of Alcohol at  $1411.14\text{ cm}^{-1}$  and C-C stretching peak of Alkane at  $1104.88\text{ cm}^{-1}$ .

### 3.8.3 $^1\text{H}$ NMR - Spectroscopy-

$^1\text{H}$  NMR spectra of isolated fraction (I) of ZO methanolic extract was recorded on NMR Spectrometer. Tetramethylsilane used as an internal standard. The signals are denoted with the symbols s, d, t, and m for singlet, doublet, triplet, and multiplet, respectively.

### (A) $^1\text{H}$ NMR spectra of the isolated Fraction (I) of ZO –

In  $^1\text{H}$  NMR spectra of isolated fraction (I) of ZO Methanolic extract showed that  $^1\text{H}$ -3 protons appeared at 0.874 ppm,  $^1\text{H}$ -6 protons appeared at 1.18-1.43 (1.25 (tt), 1.28 (dd), 1.37 (ddd) ppm),  $^1\text{H}$ -2 proton appeared at 1.53 (td) ppm,  $^1\text{H}$ -2 protons appeared at 2.46 (d) ppm,  $^1\text{H}$ -2 protons appeared at 2.62 (t) ppm,  $^1\text{H}$ -2 protons appeared at 2.87 (t) ppm,  $^1\text{H}$ -1 proton appeared at 3.55 (dd) ppm,  $^1\text{H}$ -4 protons appeared at 3.65-3.84 (3.72 (tt), 3.79 (s) ppm),  $^1\text{H}$ -3 protons appeared at 6.46-6.70 (6.53 (dd), 6.57 (dd), 6.64 (dd) ppm) and  $^1\text{H}$ -1 proton appeared at 7.28 (s) ppm.



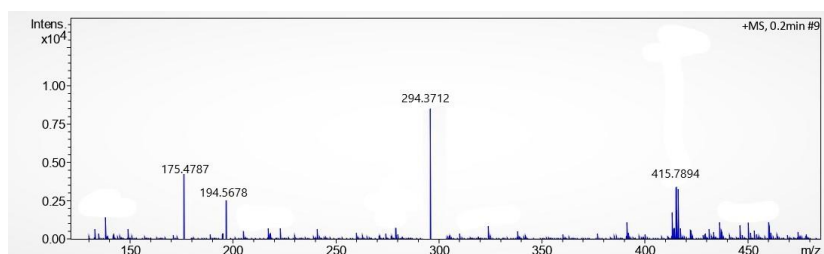
**Figure 6**  $^1\text{H}$ -NMR spectra of the isolated Fraction (I) of ZO Methanolic extract

### 3.8.4 Mass – Spectroscopy-

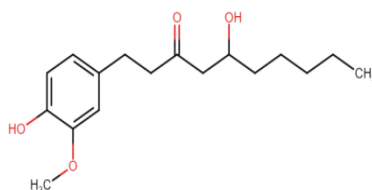
A mass spectrum of isolated Fraction (I) of ZO Methanolic extract was recorded on Mass Spectroscopy. Mass spectra of isolated Fraction (I) of ZO Methanolic extract showed molecular ion  $[\text{M}^+]$  peaks at m/z

294.3712 which obtained 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one compound in which presence of carbons ( $\text{C}_{17}$ ), Hydrogens ( $\text{H}_{26}$ ) and Oxygen ( $\text{O}_4$ ). Finally the molecular formula of isolated Fraction (I) of ZO Methanolic extract was found to be  $\text{C}_{17}\text{H}_{26}\text{O}_4$  according to their fragments.

### (A) Mass spectra of the isolated Fraction (I) of ZO Methanolic extract –



**Figure 7** Mass spectra of the isolated Fraction (I) of ZO Methanolic extract



5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one



#### 4. Discussion

The present study revealed that the total weight of *Zingiber officinale* Plant Rhizomes used were 100 gm. After performing extraction of *Zingiber officinale*, the percentage yield of Rhizome extract in different solvents like petroleum ether and methanolic extract were found to be 0.91 % (0.915 gm) and 6.08 % (6.028 gm) respectively. The methanolic extract of *Zingiber officinale* contains phytochemical constituents like carbohydrates, alkaloids, flavonoids, terpenoids, steroid, glycosides, protein & amino acid, tannins, phenolic, saponin and compounds by phytochemical investigation with respect to chemical tests. The determination of the total phenolic content, expressed as mg gallic acid equivalents and per mg/gm dry weight of sample. TPC of methanolic extract showed the content values of 187.10 mg/gm respectively. The total flavonoids content of the extracts was expressed as percentage of Rutin equivalent per mg/gm dry weight of sample. The total flavonoids content of methanolic extract showed the content values of 171.33 mg/gm respectively. In the Preliminary TLC of *Zingiber officinale* methanolic extract was performed on different solvent systems (solvent system was selected on the basis of literature survey). TLC performed in n-hexane: diethyl ether (2:3) with Std. Phenolic that was clearly visible bands in *ZO* methanolic extract. The R<sub>f</sub> value were found to be 0.48 and 0.48 of *ZO* and Std. Phenolic. (Fig 1, Table 9). So that n-hexane: diethyl ether (2:3) was taken as mobile phase for column chromatography. Active constituents are isolated from column chromatography with the mobile phase of n-hexane: diethyl ether (2:3) for *ZO* to obtained Fractions 01 (A), 02 (B), 03 (C), 04 (D), 05 (E), 06 (F), 07 (G), 08 (H), 09 (I), 10 (J) and 12 (K) (Table 10). R<sub>f</sub> value Resulted after performing the TLC estimation is also done for the confirmation of active constituents in fractions (C) of *ZO* with mobile phase n-hexane: diethyl ether (2:3) by comparing with Std. Phenolic (Fig 3, Table 11). The collected Fractions were taken properly and do the UV spectrum. UV spectra of the isolated fractions (I) of *ZO* was recorded over a scanning range of 200-800 nm and  $\lambda_{max}$  of fractions (I) of *ZO* was determined and the wavelength of *ZO*, (I) fraction was found to be 284 nm (Fig 4). The IR Spectra of isolated fraction (I) of *ZO* Methanolic extract showed that -OH group Strong, Broad peak appeared at 3459.19 cm<sup>-1</sup>, C-H stretching peaks of

Alkane at 2926.22 & 2372.80 cm<sup>-1</sup>. The Carbonyl group C=O stretching peak at 1589.97 cm<sup>-1</sup>, O-H bending peak of Alcohol at 1411.14 cm<sup>-1</sup> and C-C stretching peak of Alkane at 1104.88 cm<sup>-1</sup> (Fig 5, Table 12). In <sup>1</sup>H NMR spectra of isolated fraction (I) of *ZO* Methanolic extract showed that <sup>1</sup>H-3 protons appeared at 0.874 ppm, <sup>1</sup>H-6 protons appeared at 1.18-1.43 (1.25 (tt), 1.28 (dd), 1.37 (ddd) ppm), <sup>1</sup>H-2 proton appeared at 1.53 (td) ppm, <sup>1</sup>H-2 protons appeared at 2.46 (d) ppm, <sup>1</sup>H protons appeared at 2.62 (t) ppm, <sup>1</sup>H-2 protons appeared at 2.87 (t) ppm, <sup>1</sup>H-1 proton appeared at 3.55 (dd) ppm, <sup>1</sup>H-4 protons appeared at 3.65-3.84 (3.72 (tt), 3.79 (s) ppm), <sup>1</sup>H-3 protons appeared at 6.46-6.70 (6.53 (dd), 6.57 (dd), 6.64 (dd) ppm) and <sup>1</sup>H-1 proton appeared at 7.28 (s) ppm (Fig 6). A mass spectrum of isolated Fraction (I) of *ZO* Methanolic extract was recorded on Mass Spectroscopy. Mass spectra of isolated Fraction (I) of *ZO* Methanolic extract showed molecular ion [M<sup>+</sup>] peaks at m/z 294.3712 which obtained 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one compound in which presence of carbons (C<sub>17</sub>), Hydrogens (H<sub>26</sub>) and Oxygen (O<sub>4</sub>). Finally the molecular formula of isolated Fraction (I) of *ZO* Methanolic extract was found to be C<sub>17</sub>H<sub>26</sub>O<sub>4</sub> according to their fragments (Fig. 7)

#### 5. Conclusion

The Various examination of the *ZO* Methanolic extract plant of *Zingiber officinale* belonging to the family *Zingiberaceae* was effectively carried out. From this physical, chemical and spectral investigation were confirmed the presence of 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one in Fraction (I) of *ZO* Methanolic extract.

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