

# Physicochemical Analysis of Ginger (*Zingiber officinale* Rosc.) Rhizome along with its TLC, HPLC and HPTLC Profile

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## ABSTRACT

**Introduction:** Ginger (*Zingiber officinale* Rosc.) is a well-known traditional medicinal plant used for therapeutic effects in both Unani and Ayurvedic System of Medicine. It has been founded to have many therapeutic activities such as anti-inflammatory, anti-rheumatic and anti-gout properties. It is also useful in the management of cough, dyspepsia, loss of appetite, relaxed sore-throat, retching, spasms / spasmodic affections of the stomach, vomiting. The present study aimed towards the evaluation of the parameters involved in the determination of the quality and purity of *Zingiber officinale* Rosc. rhizome and its standardization. **Methods:** Organoleptic characters, extractive values, ash values, phyto-chemical analysis, TLC, HPTLC, fluorescence analysis and HPLC profile etc. were the parameters used for the standardisation of the test drug.

**Results:** Total ash values, water, alcohol and Ether soluble extractive values and volatile oil percentage was found to be 7.60%, 11.23%, 8.55%, 2.5% and 3.75% respectively. TLC profile of *Z. officinale* Rosc shows 05 and 13 spots in UV short and exposure in anisaldehyde-sulphuric acid respectively. The HPLC

pattern shows 29 peaks and the peak no. 2, 7 and 5 are major peaks having area concentration and retention time as 9.64% at 2.66 min, 10.12% at 3.25 min. and 15.22% at 3.61 min. respectively. **Conclusion:** The study will provide referential information for the good quality, purity and identification for the future batches of *Zingiber officinale* Rosc.

**Key words:** Phyto-chemical analysis, Quality, Standardization, HPTLC, Unani.

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## INTRODUCTION

Nowadays the Indian herbal industry is focusing on making an effort with remarkable increase in the introduction of new herbal pharmaceutical and cosmetic products in the market. But due to insufficient, spurious and adulterated supply of herbal medicines, quality and purity of herbs and their products is not assured. As the potency, efficacy and safety of herbal products are mainly based on their good quality therefore, identification, quality and purity of the herbal medicines are necessary. The traditional standardization methods of herbal medicines are not sufficient, now more physical and analytical advanced techniques are required. Standardization of the crude drugs involves passport data of the drugs viz., botanical identification, macroscopic, microscopic and molecular examination, identification of Phytochemical constituent by various chromatographic techniques and biological activity of the whole plant.<sup>1</sup>

As the rhizome of *Zingiber officinale* Rosc. is often contaminated and adulterated with different plant materials such as white and black pepper, Rasen, Pellitory.<sup>2</sup> Therefore, in this study *Zingiber officinale* Rosc., family – Zingiberaceae was selected and standardized on their physico-chemical characteristics along with TLC, HPTLC and HPLC profile. *Zingiber officinale* is native of tropics and widely cultivated in tropical Asia. At present it is cultivated in all the warmer regions of whole world notably in the West Indies, Nigeria, China and India and commonly known as Adrak.<sup>3</sup> It is an excellent remedy popular to the Indian medicinal system and is used from centuries for its health benefits; it can also be used for Appetiser, Carminative. Medicinally it possesses Anti-inflammatory, Antiemetic, Antiulcer, Stimulant, Antiplatelet, Antibacterial, Antifungal properties and can be used in Sialagogue, Alexiteric, Anthelmintic / Vermifuge, Aphrodisiac, Diuretic, Expectorant, Improves taste, Laxative, Stomachic, Tonic, Digestive, Laxative, Stomachic etc.<sup>4</sup> The oil of ginger is a mixture of constituents, consisting of monoterpenes (phellandrene, camphene,

cineole, citral and borneol) and sesquiterpenes (zingiberene, zingiberol, zingiberenol,  $\beta$ -bisabolene, sesquiphellandrene and others). Aldehydes and alcohols are also present. 10-gingerol, 12-gingerol, 8-shogaol, 10-shogaol, 6-gingerdione, 8-gingerdione, 10-gingerdione, 6-dehydro-10-gingerol, 6-paradol and 8-paradol are also present in extract of ginger. The levels of constituents, representing 96% of the components of Ginger Essential Oil (GEO) are: ar-curcumenol (59%), b-myrcene (14%), 1,8-cineol (8%), citral (7.5%) and zingiberene (7.5%).<sup>5</sup>

The volatile oil components in ginger consist mainly of sesquiterpene hydrocarbons, predominantly zingiberene (35%), curcumenol (18%) and farnesene (10%), with lesser amounts of bisabolene and b-sesquiphellandrene. A smaller percentage of at least 40 different monoterpenoid hydrocarbons are present with 1, 8-cineole, linalool, borneol, neral and geraniol being the most abundant.<sup>6</sup>

Many of these volatile oil constituents contribute to the distinct aroma and taste of Ginger. Non-volatile pungent compounds: This species contains biologically active constituents including the non-volatile pungent principles, such as the gingerols, shogaols, paradols and zingerone that produce a “hot” sensation in the mouth. The gingerols, a series of chemical homologs differentiated by the length of their unbranched alkyl chains, were identified as the major active components in the fresh rhizome. In addition, the shogaols, another homologous series and the dehydrated form of the gingerols, are the predominant pungent constituents in dried ginger. Paradol is similar to gingerol and is formed on hydrogenation of shogaol. Other constituents: In addition to the extractable oleoresins, Ginger contains many fats, waxes, carbohydrates, vitamins and minerals. Ginger rhizomes also contain a potent proteolytic enzyme called zingibain.<sup>7</sup>

6-Shogaol is one of the major compounds in the ginger rhizome that may contribute to its anti-inflammatory properties. In a study, Sprague-Dawley rats (200–250 g) treated with a single injection (0.5 ml of 1 mg/ml) of a commercial preparation of Complete Freund's Adjuvant (CFA) to induce mono-

arthritis in the right knee over a period of 28 days. 6-shogaol reduced the inflammatory response and protected the femoral cartilage from damage produced in a CFA monoarthritic model of the knee joint of rats.<sup>8</sup>

The present study was carried out in order to standardise the rhizome of *Zingiber officinale* Rosc. with its quality parameters development and also to deliver essential information for the identification of the crude drug so as to check the substitution and adulteration and to ensure the effectiveness of a drug in treating different body ailments. Parameters include macroscopy, powder analysis, physicochemical parameters and preliminary phyto-chemical screening along with HPLC and HPTLC profile.<sup>9</sup>

## MATERIALS AND METHODS

### Collection of Sample

Dried rhizome of *Zingiber officinale* Rosc. was procured from local market of Aligarh and was properly recognized from the accessible literature and authenticated by Prof. Abdul Latif. The sample with specimen voucher no. SC-0226/17 was deposited in the museum of Department of Pharmacy, Faculty of Unani medicine, Aligarh Muslim University, Aligarh, for future reference. It was crushed and sieved to coarse powder mechanically and stored in air tight container for study.

#### Macroscopy and Organoleptic Characters

The organoleptic characters of the crude drug were observed with sensory organs and was analysed for its colour, odour and taste, size, shape, fracture and surface.<sup>9-11</sup>

#### Physicochemical Parameters

Ash values, alcohol and water-soluble extractive values, volatile oil estimation of the test drug was determined as per the methods recommended by Ayurvedic Pharmacopoeia of India (API) and British Pharmacopoeia.<sup>12-14</sup>

The fluorescence analysis of the rhizome powder was done by treating with the different chemical reagents and observed under Ultra violet light and day light.<sup>15,16</sup>

#### TLC

Thin layer chromatographic analysis of the methanolic extract of *Zingiber officinale* Rosc. was carried out via Toluene: Ethyl Acetate: Formic Acid (9:1:2 drops) and n-Hexan: Ethyl Acetate (9.5:0.5) as mobile phase in percolated silica gel 60F<sub>254</sub> TLC plates. Spotted TLC plates were sprayed by Anisaldehyde- sulfuric acid and were also visualized in day light and UV short and long wavelength. The  $R_f$  value of spots was determined by the given formulae.<sup>14,17</sup>

$R_f$  Value = Distance travelled by the spot / Distance travelled by the solvent

#### Preliminary Phyto-Chemical Screening

The extracts were introduced to preliminary phyto-chemical analysis and investigated for the presence of various phyto-constituents like alkaloids, carbohydrates, glycosides, flavonoids, proteins, steroids, saponins, etc. with following parameters.<sup>18,19</sup>

### HPLC Profile of *Zingiber officinale* Rosc.

HPLC profile of the methanolic extract of the *Zingiber officinale* Rosc. was done. For this Shimadzu Prominence Isocratic HPLC System equipped with LC-20 AD Solvent delivery unit, Rheodyne Injector, SPD-20A prominence Uv-vis detector system along with C<sub>18</sub> G120A column, 250 x 4.6 mm 5U with guard column was used. The methanolic extract of coarsely powdered drug was obtained with the help of Soxhlet's extraction method, extract was filtered and allowed to evaporate on water

bath. This dried alcoholic extract was dissolved in HPLC grade methanol and used for study. The chromatographic analyses were carried out at room temperature using reversed phase and software driven peaks were obtained (Figure 2). The pressure and flow rate was 127 kgf and 1.0 ml/min, respectively. Detector for HPLC was UV and the wavelength was 254 nm. Mobile phase for HPLC profile of extract consisted of HPLC grade methanol (Merk life science Pvt. Ltd.) only.

## RESULTS AND DISCUSSION

Modern system of medicine has sound experimental data, toxicity studies and human clinical studies. But in herbal medicine, there is a lack of pharmacopoeial standards on raw material / finished products. The insufficient quality standards have led to the occurrence of mild to serious adverse effects. Hence, the standardization of herbal ingredients is the basic requirement in order to establish the identity, purity and quality.<sup>20</sup> Herbals are traditionally considered safe and are remarkably consumed by people without prescription. However, it is observed that some can cause health problems, some are not effective and some may interact with other medicines. Standardization is essential for the assessment of the quality, purity and authenticity of the drugs, based on the physicochemical parameters, TLC, HPLC and HPTLC on the presence of active principles.<sup>21</sup> A standardized, authenticated good quality and purity drug is the assurance of its therapeutic effectiveness and global acceptance. *Zingiber officinale* Rosc. is a well-known drug of Unani System of Medicine used to treat various body ailments such as inflammatory and rheumatic conditions. Therefore, for this study *Zingiber officinale* Rosc. was selected and standardized on their physicochemical parameters such as organoleptic characters, ash values, extractive values, volatile oil estimation, fluorescence analysis, Phytochemical study, qualitative estimation, TLC along with HPLC and HPTLC profile.

### Organoleptic characters of *Zingiber officinale* Rosc.

Organoleptic properties are the critical parameter for the rapid identification and consumer acceptance. Sensory evaluation-visual macroscopy, colour, odour, taste, fracture are the common features helped in identification of the crude drug. The organoleptic properties of rhizome of *Zingiber officinale* Rosc. have been mentioned in Table 1.

### Physicochemical analysis of *Zingiber officinale* Rosc.

Ash values, alcohol and water-soluble extractive values, in powdered drug are the indicators of the purity, quality and authenticity of any crude drug. Therefore, to standardise a herbal drug these parameters have basic importance and unavoidable. Total ash values, acid insoluble and water-

**Table 1: Organoleptic Characters of *Zingiber officinale* Rosc.**

Rhizome of <i>Zingiber officinale</i> Rosc.	Characters
Shape	Nodule
Size	Rhizomes are 2.5 to 7.5 cm in length
Colour	Dark grey rhizomes are crowned with brownish Soft fibres.
Fracture	Easy and splintery
Surface	Scaring
Odour	characteristic odour
Taste	sharp taste Pungent like <i>Piper nigrum</i>

soluble ash values reveals the information related to the adulteration of crude drug with inorganic matter. The water and alcohol soluble extractive values indicate the amount of the extract that the drug yields in a solvent.<sup>16</sup> Less or more extractive value indicates that there is addition of exhausted material, adulteration or incorrect processing during drying or storage of plant products.<sup>22</sup> Low or high moisture contents may affect the quality as well as its efficacy of the drug. The excessive moisture is an ideal medium for the growth of the different types of microorganisms which subsequently damages the drug.<sup>23</sup> This drug is also well known for its oil contents. The inappropriate method of extraction of oil or distillation and storage may spoil the quality of the drug and hence the oil.<sup>23</sup> Therefore, to assess the quality of *Zingiber officinale* Rosc. it is also necessary to determine volatile oil percentage of the drug. All the values were determined in triplet and the results are depicted in Table 2. Successive extractive values of powdered drug in different solvents viz. petroleum ether, diethyl ether, chloroform, benzene, alcohol, methanol and distilled water were also determined with Soxhlet's apparatus. The extractive values were expressed in percentage and depicted in Table 3.

### Fluorescence analysis of *Zingiber officinale* Rosc.

Some constituents in many natural products exhibit fluorescence in the daylight ultra violet light and if the substance itself is not fluorescent, it may often be converted into fluorescent through the application of different reagents. Hence, the qualitative assessment of the test drug is carried out in this manner also which serves as an important parameter for pharmacognostic evaluation of crude drugs.<sup>24</sup> (Table 4)

### Phytochemical Analysis of *Zingiber officinale* Rosc.

The efficacy and pharmacological therapeutic effects of any herbal medicine is depends on their secondary metabolites i.e. phytoconstituents such as alkaloids, terpenoids and glycosides etc. The presence or absence of these phytoconstituents also indicates the quality of the crude drug.<sup>24</sup> Therefore, it is also necessary to determine the presence of active secondary metabolites in the test drug, the results are shown in Table 5.

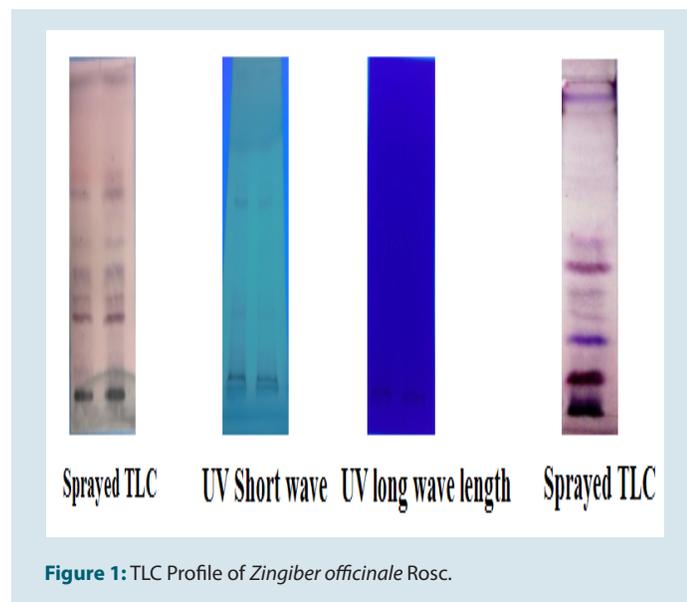


Figure 1: TLC Profile of *Zingiber officinale* Rosc.

### TLC of *Zingiber officinale* Rosc.

TLC is one of the important parameter equips with the qualitative and semi-quantitative information of the drug. If the drug is adulterated or exhausted which in turn may increase or decreases the number of spots and change in the  $R_f$  values.<sup>16</sup> The TLC profile along with images of TLC are illustrated in Table 6 and Figure 1 respectively

### HPLC Profile of Methanolic Extract of *Zingiber officinale* Rosc.

The preparative and analytical HPLC has been widely employed for the analysis of herbal medicines in lieu of its high separation capacity. It can also be utilized to analyse almost all constituents of herbal products provided that an optimized procedure is developed which involves optimization of mobile phase and stationary phase along with other chromatographic parameters.<sup>25</sup> The adulteration and impurities can also be determined by this technique. If there is any change in number of peaks or retention time or area of peaks from standard it indicates adulteration or deterioration in the drug. The HPLC pattern shows 29 peaks and the peak no. 05 and 07 are major peaks having area concentration and retention time as 10.302% at 3.250 min. and 15.228% at 3.612 min. respectively followed by peak no. 02, 03, 08, 19 and 10 with concentration of 9.641%, 9.037%, 7.538%, 7.190% and 7.113% respectively. The HPLC profile of the test drug was obtained and recorded for future reference. The details are depicted in Figure 2 and Table 7.

### HPTLC profile of Hydroalcoholic extract of *Zingiber officinale* Rosc.

The preparative and analytical HPTLC has been widely employed for the analysis of herbal medicines due to its high separation capacity. It can also be utilized to analyse almost all constituents of herbal products provided that an optimized procedure is developed which involves optimization of mobile phase and stationary phase along with other chromatographic parameters.<sup>26</sup> The adulteration and impurities can also be determined by this technique. If there is any change in number of peaks or  $R_f$  Value or area of peaks from standard it indicates adulteration or deterioration in the drug. The HPTLC pattern shows 09 peaks and the peak no. 08 and 06 are major peaks having area concentration and  $R_f$  Value as 60.19% at 0.83  $R_f$  and 15.10% at 0.69  $R_f$ , respectively followed by peak no.

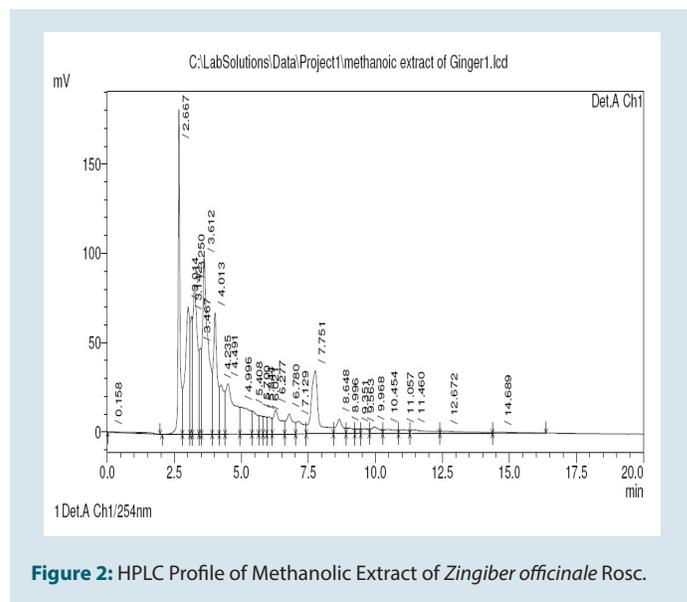


Figure 2: HPLC Profile of Methanolic Extract of *Zingiber officinale* Rosc.

**Table 2: Physico-Chemical Characters of *Zingiber officinale* Rosc**

S. No.	Physico-Chemical Parameters	Result (Mean± SEM)
1.	Water soluble matter (%)	11.233± 0.4651
2.	Alcohol soluble matter (%)	8.55± 0.1923
3.	Ether soluble matter (%)	2.5± 0.0577
4.	Total Ash (%)	7.6083± 0.1491
5.	Acid Insoluble Ash (%)	1.900± 0.0577
6.	Water Soluble Ash (%)	3.366± 0.3609
7.	Volatile Oil (%)	3.75± 0.0404
8.	Total Alkaloid (%)	5.36± 0.0288
9.	pH (1% solution)	7.20± 0.2309
10.	pH (10% solution)	7.20± 0.2309

**Table 3: Successive Extractive Values of Formulation.**

S. No.	Solvent	Extractive values in % (Mean± SEM)
1.	Petroleum Ether	2.5±0.1155
2.	Toluene	3.50±0.0577
3.	Chloroform	0.52±0.0057
4.	Acetone	0.40±0.01732
5.	Methanol	5.6±0.1155
6.	Acetonitrile	0.26±0.0115
7.	Distilled water	12.92±0.03464

**Table 4: Fluorescence Analysis of *Zingiber officinale* Rosc.**

Reagents	Visible light	UV light	
		Short 254nm	Long 366
Powder as such	Brown	Brown	Brown
Powder+ 1N HCl	Light Brown	Light Brown	Indigo
Powder+50% H <sub>2</sub> SO <sub>4</sub>	Brown	Light green	Dark Brown
Powder+50% HNO <sub>3</sub>	Pinkish	Greenish yellow	Indigo
Powder+ Glacial acetic acid	Brown	Light green	Grey
Powder+1N NaOH in water	Dark Brown	Dark greenish	Black
Powder+1N NaOH in methanol	Brown	Dark greenish	Violet
Powder+ Wagner's reagent	Brown	Brown	Indigo
Powder+ Dragendorff reagent	Yellow	Green	Indigo
Powder+ Benedict's reagent	Brown	Brown	Indigo
Powder+ Fehling reagent	Brown	Brown	Indigo

**Table 5: Phytochemical analysis of *Zingiber officinale* Rosc.**

Chemical Constituents	Test / Reagents	Extract	Inference
<b>Alkaloids</b>	Dragendorff's reagent	Aqueous	Present
	Tannic acid test	Aqueous	Present
<b>Carbohydrates</b>	Fehling's test	Aqueous	Present
	Molish's test	Aqueous	Present
<b>Flavonoids</b>	Alkaline reagent test	Aqueous and Alcoholic	Present
<b>Proteins</b>	Biuret test	Aqueous	Present
<b>Glycosides</b>	General Test	Aqueous	Present
<b>Saponins</b>	Frothing with NaHCO <sub>3</sub>	Aqueous and Alcoholic	Present
	Copper sulphate / Sodium hydroxide	Petroleum	Present
<b>Fats and fixed oils</b>	Sodium bisulphate test	Petroleum	Present
<b>Terpenoids</b>	Sulphur powder sink test	Aqueous and Alcoholic	Present
<b>Starch</b>		Aqueous	Present

**Table 6: TLC Profile of Methanolic Extract of *Zingiber officinale* Rosc.**

Spray / Light treatment	Toluene: Ethyl Acetate: Formic Acid (9:1:2 drops)		n-Hexan: Ethyl Acetate (9:1)	
	No. of Spots	R <sub>f</sub> Values and Colour of spots	No. of Spots	R <sub>f</sub> Values and Colour of spots
<b>UV Short wave length</b>	5	0.02(G), 0.06(Gr), 0.10(As), 0.35(BL), 0.42(As)	No spot	
<b>UV long wave length</b>	No spot		No spot	
<b>Anisaldehyde-sulphuric acid</b>	13	0.02(G), 0.04(V), 0.06(Gr), 0.08(BL), 0.10(As), 0.14(B), 0.18(BL), 0.20(As), 0.35(BL), 0.42(As), 0.57(B), 0.62(Gr), 0.97(As), 0.61(B)	10	0.02(Br), 0.08(DB), 0.20(V), 0.28(B), 0.32(LB), 0.35(LV), 0.42(Br), 0.52(P), 0.91(G), 0.95(BL)

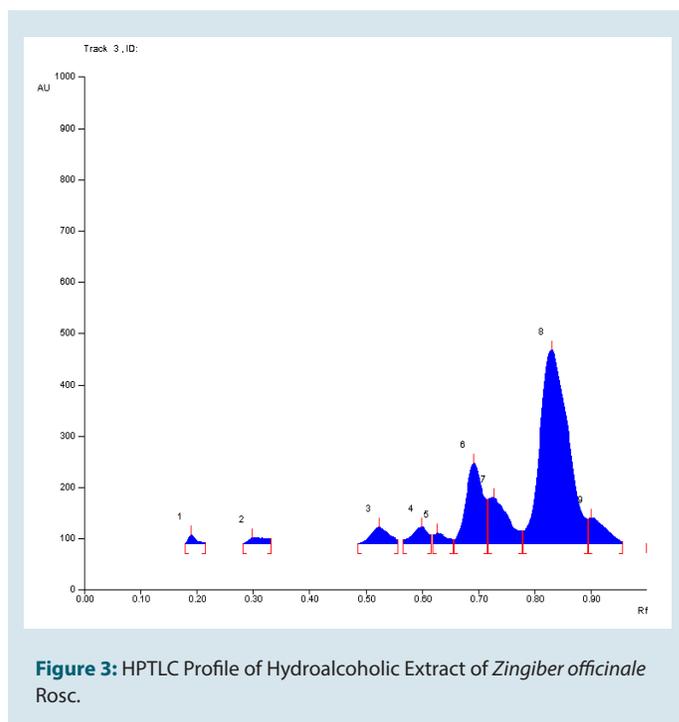
Note: V=Violet, LV=Light Violet, P=Pink, Br=Brown, BL=Blue, DB=Dark Brown, LB=Light Brown B=Black, G=Green, DG=Dark Green, Gr= Gray, As= Ash Colour.

Table 7: HPLC Profile of Methanolic Extract of *Zingiber officinale* Rosc.

Peak	Retention Time	Height	Area	Height %	Area %
1	0.158	280	43975	0.034	0.474
2	2.667	181743	894680	21.963	9.641
3	3.014	71212	838611	8.606	9.037
4	3.142	65897	355463	7.964	3.830
5	3.250	83762	956011	10.123	10.302
6	3.467	48115	262118	5.815	2.824
7	3.612	98355	1413200	11.886	15.228
8	4.013	68012	699494	8.219	7.538
9	4.235	27610	333761	3.337	3.596
10	4.491	28243	660068	3.413	7.113
11	4.996	15137	367600	1.829	3.961
12	5.408	12918	179725	1.561	1.937
13	5.700	10369	101664	1.253	1.095
14	5.844	9921	82545	1.199	0.889
15	6.021	9676	111341	1.169	1.200
16	6.277	13553	257813	1.638	2.778
17	6.780	11212	201158	1.355	2.168
18	7.129	7008	140909	0.847	1.518
19	7.751	35387	667204	4.276	7.190
20	8.648	8153	138198	0.985	1.489
21	8.996	3198	59742	0.386	0.644
22	9.351	2783	35845	0.336	0.386
23	9.563	2813	54964	0.340	0.592
24	9.968	3828	83752	0.463	0.902
25	10.454	2427	75126	0.293	0.810
26	11.057	2063	48226	0.249	0.520
27	11.460	2050	96341	0.248	1.038
28	12.672	1194	90134	0.144	0.971
29	14.689	561	30515	0.068	0.329
Total		9280185	9280185	100.000	100.000

Table 8: HPTLC Profile of Hydroalcoholic Extract of *Zingiber officinale* Rosc.

Peaks	R <sub>f</sub> Value	Area %
1	0.19	0.75
2	0.30	1.25
3	0.52	3.30
4	0.60	2.90
5	0.63	1.54
6	0.69	15.10
7	0.73	10.05
8	0.83	60.19
9	0.90	4.92

Figure 3: HPTLC Profile of Hydroalcoholic Extract of *Zingiber officinale* Rosc.

07, 09 and 03 with concentration of 10.05%, 4.92% and 3.30% respectively. The HPTLC profile of the test drug was obtained and recorded for future reference. The details are depicted in Figure 3 and Table 8.

## CONCLUSION

Present study shows that the methods of standardization and identification of *Zingiber officinale* Rosc. i.e. organoleptic characters along with physico-chemical analysis are the basic and useful parameters to analyse the originality of the test drug. A good quality of drug is the assurance of its efficacy. The results of phytochemical analysis and HPLC fingerprinting also play a key role in identification and authentication of *Zingiber officinale* Rosc. Further these analytical parameters for quality assurance

also indicating effectiveness of *Zingiber officinale* Rosc. for treating various body ailments. The data obtained in the present work will be useful in identification, standardisation and quality assurance of different samples of *Zingiber officinale* Rosc. and will also be useful in the preparation of the drug's monograph for inclusion in various pharmacopoeias.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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## PICTORIAL ABSTRACT



Plant of Ginger



Dried Rhizome of Ginger

## SUMMARY

- The present study aims towards the evaluation of the parameters involved in the determination of the quality and purity of *Zingiber officinale* Rosc. rhizome and its standardization. The parameters used for the standardisation of the test drug includes organoleptic characters, extractive values, ash values, phyto-chemical analysis, TLC, fluorescence analysis and HPLC profile etc. The study will provide referential information for the good quality, purity and identification for the future batches of *Zingiber officinale* Rosc.

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