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Quality standard, lipid profile and anti-lipase studies of *Zangiber officinale*

Md. Rafiul Haque, SH Ansari and Mahfuz Alam

Abstract
Zangiber officinale, commonly called as ginger, its rhizome part is a very important for its traditional uses due to presence of oleoresin and volatile oil. It belongs to the family Zangiberaceae. The objectives of current study are to determine the quality and evaluation of anti-lipase activity and lipid profile in *Zangiber officinale* on obese rat modal. Determination of quality of *Zangiber officinale* Roscoe rhizome is done with the help of microscopical and physiochemical studies. The composition of ginger oil was analyzed by gas chromatography (GC) and gas chromatography mass spectroscopy (GCMS). *Zangiber officinale* distillate (ZD) was prepared for the study of lipid profile and pancreatic lipase activity. All these physiochemical values and microscopical observations were under pharmacopeia's limits and observation. Volatile oil content was 1.2 ± 0.03%w/w, and 53 components were identified. Camphene (18.28%), para-cineole (10.02%), zingiberene (6.12%), and cuminaldehyde (2.17%) were as major components. After end of the treatment, *Zangiber officinale* distillate (7.75 ml/kg, twice a day) treatments showed significant reduction in serum lipids. But ZD did not significantly alter the pancreatic lipase activity. These results recommend that ZD have strong hypolipidemic activity without inhibition of pancreatic lipase in HFD induced obese rat model.

Keywords: *Zangiber officinale*, pancreatic lipase, pesticide, toxic metal, GCM

1. Introduction
Zingiber officinale Roscoe is widely used as spice and medicinal plant in folk and traditional medicines. Ginger oil is isolated from *Z. officinale* rhizomes, which its chemistry affected from geographical factors, separation methods, freshness or dryness of rhizomes [1]. Ginger contains proteins (9%), carbohydrates (60-70%), crude fiber (3-8%), ash (8%), fatty oils (3-6%), water (9-12%), and volatile oil (2-3%). Zingiberene + zingiberol (38.9%), ar-curcumene (17.7%), β-sesquiphellandrene+β-bisabolene (11%), β-phellandrene (4.9%) were the main components of simultaneous distillation extracted ginger oil from fresh rhizome (Bangalore market) [2]. The essential oil from unpeeled rhizomes cultivars from North-East India (2.22-4.17% w/w) had camphene (8.49%), neral (4.95%), geranial (12.36%), zingiberene (20.98%) and β-sesquiphellandrene (7.96%) [3]. The chemistry of ginger oils are affected from geographical factors. Ginger essential oils, separated by hydro-distillation method from three different geographical region of India (Mizoram, Chennai and two varieties from Sikkim) had zingiberene (10.5-16.6%), ar-curcumene (2.9-9.8%), β-sesquiphellandrene (5.8-7.2%), E-citral (7.4-10.5%), Z-citral (5.3-7%), o-cymene (0.9-6.5%), camphene (0.9-7.6%), and limonene (1.3-6.4%) [4] as a major chemical constituents. For the management of different types of disease, ginger plant has been used from the primitive times [5]. It has been investigated that ginger has various biological activities such as, immunomodulatory [6], antioxidant [7], anti-inflammatory [8], antidiabetic [9-12], anticancer [13], Hypolipidemic [14], anti-oxidative stress [15], analgesic [16] and antihyperlipidemic effects [17]. Plants contain various types of an active chemical component that can be used for the therapeutic purpose or as precursors for pharmaceutical synthesis. During recent times, herbal therapies have become more popular due to low cost, more effectiveness, easy availability, and fewer adverse effects. As a result, global market demand for herbs and their products has increased tremendously in current years. Therefore, quality control or evaluation of herbal products for the purpose of efficacy and safety is essential. Authentication and development of quality control parameters are essential for any crude drug and their formulation [18]. The World Health Organization (WHO) and Unani Pharmacopeia of India (UPI) have described a number of quality control parameters for determining the quality and purity and confirming for identity of crude drug and herbal products. Quality control is determined on the basis of identity, purity, quality by organoleptic,

microscopical, chemical, physical, and/or biological properties, as well as by manufacturing process [19-20]. Thus, in this research work attempt has been made for standardization of *Zangiber officinale* rhizome by evaluating its microscopical and physicochemical properties. This study was designed to explore the hypolipidemic and pancreatic lipase activity of ZD using high fat diet (HFD) rat model.

2. Materials and Methods

2.1 Plant materials

The rhizome of *Zingiber officinale* Roscoe was obtained from raw drug supplier, Ballimaran, New Delhi, (India) with the knowledge of Unani physician at the month of December. The rhizome was authenticated from National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. Voucher specimen certificate reference number NISCAIR/RHMD/Consult/2011-12/1753/53 was received and reserve in the department for future reference.

2.2 Macroscopic and microscopic characters

Detailed study of the morphological characters was helpful in differentiating the crude drugs. The macroscopic characters (size, color, odor and taste) of a drug were observed by its visual appearance with help of naked eye. Microscopically characters (cork, cortex, starch grain, oleoresin, endodermis and crystals etc of a drug of transverse section microscopy was observed by the help of Motic (a microscope fitted with computer).

2.3 Determination of foreign mater

Weighed 100-500 g of the drug sample and spread it out in a filter paper. Separated the foreign matters and weighed it and calculated the percentage present [20].

2.4 Determination of total ash

Incinerated about 2 to 3 g of powdered drug accurately weighed in a tared platinum or silica dish at a temperature not exceeding 450° until free from carbon, cooled and weighed and finally calculated the percentage of ash with reference to the air-dried drug [20].

2.5 Determination of acid-insoluble Ash

Boiled the ash obtained in 2.4 section for 5 minutes with 25 ml of dilute hydrochloric acid; collected the insoluble matter in a Gooch crucible or on an ash less filter paper, washed with boiled water and ignite to constant weight. Lastly calculated the percentage of acid-insoluble ash with reference to the air dried drug [20].

2.6 Determination of water-soluble ash

Boiled the ash with 25 ml of water for 5 minutes and collected insoluble matter in a crucible, or on as ash less filter paper, washed with boiled water, and ignite to constant weight. Lastly minus the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash and calculated the percentage of water-soluble ash with reference to the air-dried drug [20].

2.7 Determination of alcohol-soluble extractive

Dissolved 5 g powdered drug with 100 ml of ethyl alcohol in a closed flask for 24 hours, shaking frequently during 6 hours and allowing standing for 18 hours. Filtered, and evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°, to constant weight and weighed and calculated the percentage of alcohol-soluble extractive with reference to the air-dried drug [20].

2.8 Determination of water-soluble extractive

Dissolved 5 g coarsely powdered with 100 ml of distilled water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filtered and evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°, to constant weight and weighed and calculated the percentage of water-soluble extractive with reference to the air-dried drug [20].

2.9 Determination of volatile oil of crude drugs

The determination of volatile oil in a drug was fined out by distilling the drug with a mixture of water and glycerin, collecting the distillate in a graduated tube in which the aqueous portion of the distillate was automatically separated and returned to the distilling flask, and measured the volume of the oil. The content of the volatile oil is expressed as a percentages v/w [20].

2.10 GCMS and GC analysis of volatile oil

For the analysis of the oil, test sample injected on a Shimadzu QP-2010 GC-MS system equipped with AB-Innowax 7031428 WCOT column (60 m x 0.25 mm x 0.25 µm) directly coupled to the MS. The carrier gas was He(helium) with a flow rate of 1.21 ml/min. oven temperature was programmed as 50 ºC for 1 min and subsequently held isothermal for 2 min. injector port: 250 ºC, detector: 280 ºC, split ratio 1: 50, volume injected: 1 µl of the oil. The recording was determined at 70 eV, scan time 1.5 s and mass range 40-750 amu. Software adopted to handle mass spectra and chromatograph was a Chem station and gas chromatographic analysis of the oil was fined out on Shimadzu 2010 gas chromatograph (Japan) equipped with a flame ionization detector and AB-Innowax 7031428 WCOT fused column (60 m x 0.25 mm x 0.25 µm). The injector and detector (FID) temperatures were maintain at 250 and 270 ºC, respectively. The carrier gas was used as nitrogen (N_2) at a flow rate of 1.21 ml/min with column pressure of 155.1 kPa. The test sample (0.2 µl) was injected into the column with a split ratio of 80:1. Component separation was achieved following a linear temperature programmed from 60-230 ºC at a rate of 3 ºC/min and then held at 230 ºC for 9 min, with a total run time of 55.14 min. Percentage of the constituents were calculated by electronic integration of FID peak areas [21]. GC and GCMS chromatogram of ginger oil is shown in figure 1and 2.

2.11 Toxic chemical study

Analysis of aflatoxons, toxic metals and pesticide residue were performed according to procedure followed by Haque *et al*. 2012 [18].

2.12 Preparation of ZD

Zangiber officinale (R.) rhizome were cleaned with tap water to remove salt and dried in an air dryer at 37 °C for 40 h. Then it was ground into the coarse powder ant it stored at −20 °C until used. The dried coarse powder of *Zangiber officinale*. (1000 g) was soaked in distilled water and transferred to the distillation plant along with distilled water (12 L). This was distilled at 100 °C for about five and half h and 4 L of distillate (ZD) was collected.

2.13 Experimental Animals
Wistar Albino male rats, weighing 150 - 200g (8-12 weeks old), were obtained from the Central Animal House Facility, Hamdard University, New Delhi, India. Before the beginning of the experiment, the rats were accustomed for one week to the laboratory circumstances. They were maintained in polycarbonate cages, under restrained temperature (25 ± 2 °C) and 12 h light/12 h dark rhythm. The rats were permitted free access to normal pellet diet and water *ad libitum*. The procedure employed in this experiment for the use of rats was approved by the Institutional Animal Ethics Committee (IAEC) of Hamdard University (Approval letter number – 173/GO/Re/S/2000/CPCSEA, dated—28 January 2000).

2.14 Kits, chemical and experimental diets
Total cholesterol (TC), total triglyceride (TG) (Span Diagnostics Ltd, Surat, Gujarat, India), lactate dehydrogenase (LDH), high-density lipoprotein-cholesterol (Reckon Diagnostics Pvt Ltd, Baroda, Gujarat, India), Quanti ChromTM Lipase Assay Kit (DLPS-100) were purchased from different suppliers for the purpose of serum biochemical study. Normal pellet diet (NPD**)** is consisting 12.5% fat, 62.3% carbohydrate and 24.3% protein) which was purchased from Amrut rat feed, Mfd by: Pranav Agro Industries Ltd, Maharashtra, India. HFD (60% fat, 20% protein, and 20% carbohydrate) was purchased from National Centre for Laboratory Animal Science (NCLAS), National Institute of Nutrition (NIN), Hyderabad, India. All other chemicals used were of analytical grade. Double purified water was used for all biochemical measurement.

2.15 Treatments schedule and serum biomarkers measurements
After one week of acclimation, rats were then randomized into 4 groups (n = 6/group).

Group1 (Control group): The animals were fed *ad libitum* for 2 weeks. After 2 weeks, 0.5ml CMC, the vehicle was administered orally along the same diet for 4 weeks.

Group 2 (HFD group): The animals were fed on HFD orally for 6 weeks.

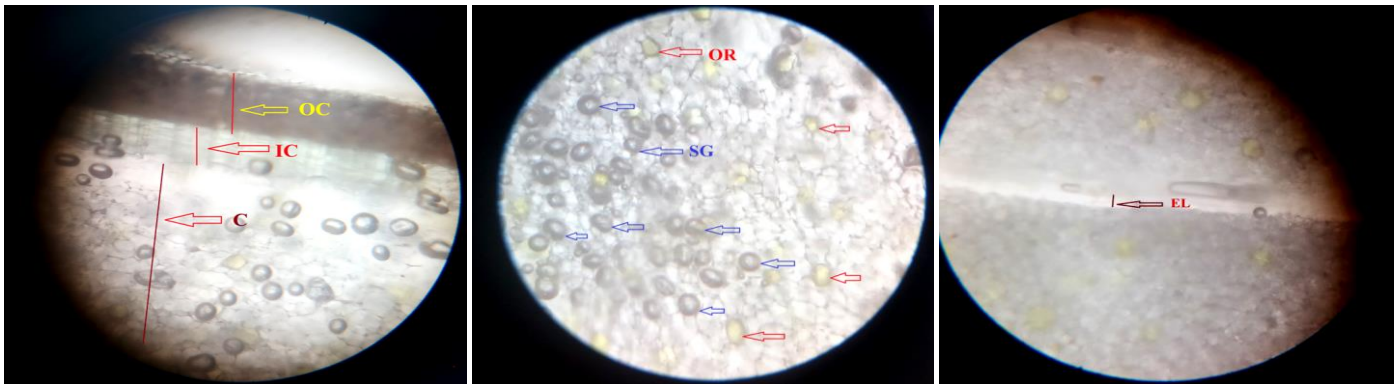
Groups 3 (HFD + ZD): Animals were received HFD for two weeks. After two weeks, along with HFD, AA (7.5 ml/kg, twice a day) was given orally (by gavage) for 28 days

Groups 6 (HFD + orlistat): Animals were received HFD for two weeks. After two weeks, along with HFD, orlistat (30 mg/kg, once a day) was given orally (by gavage) for 28 days.

Normal control group received 0.5% carboxy methyl cellulose (CMC) sodium aqueous solution. ZD was suspended in 0.5% carboxymethyl cellulose sodium aqueous solution for animal's treatment. Body weights were monitored every week. At the end of the treatment period, rats were anesthetized with ether, following a 12-h period of fasting. Blood was drawn from the retro-orbital plexus into centrifuged tubes, and the serum was obtained by centrifuging the blood at 4000 rpm for 10 min. The serum samples were stored at −70 °C until they were analyzed. Serum TC, TG, HDL-C, and pancreatic lipase activity in serum were measured with commercial kits. Assessment of serum low-density lipoprotein-cholesterol (LDL-C) cholesterol was measured by the using of standard method.

3. Results and Discussions
Adulteration (particularly in aromatic plants) is one of the major problems due to the absence of standards of the proper identification, authentication and quality control of the crude drug. Skill hand and cost factors for pharmaceuticals purposes, the quality of crude drug must be as high as that of other herbal preparations. Quality refers to the intrinsic value of the crude drug, the number of active principles or active constituents present. This study of microscopical and physicochemical of ginger rhizome revealed a set of parameters which may enable those who handle this plant to validate and maintain its quality control.

3.1 Microscopical evaluation
Microscopical examination of crude drug is very important parameter for identification and authentication of crude drug. The ginger rhizome showed the presence of outer and inner cork cell, cortex, starch grain, oleoresins and endodermis etc. The outer cork was thick layer while inner cork was thin layer. Cortex was made up various layers of parenchymatous cell and found just below the inner cork layer. Starch grain (light black) and oleoresin (light yellow) were also observed in the cortex region. An endodermis (single layer) was observed in between the cortex region. The transverse section of ginger is shown in figure 1. The outcomes obtained in microscopy studies reported herein established the microscopic parameters that characterize the genuine ginger rhizome. These microscopic characteristics can be utilized for identification and purity of the crude drug and are particularly useful in the case of marketed rhizome materials.



A B C

Fig 1: Transverse section of ginger rhizome (OC- Ourter cortex, IC- Inner cortex, C- cortex, OR- oleoresins, SG- starch grain, EL- endodermis layer)

3.2 Physicochemical evaluation

The physicochemical evaluation of crude drug is useful to determine the physiological and non-physiological adulteration. Evaluation of physical constant of any crude drug is important for the detection of adulteration and improper storage or handling. The total ash value indicates the level of physiological and non-physiological ash (inorganic materials such as silicates, carbonate and earthy materials) in the crude drug. The insoluble ash value gives the idea about adulteration of the drug with the inorganic materials [22]. The result of total ash value found in the rhizome was 5.44% (w/w). The result acid insoluble ash value was 0.5%. Alcohol soluble and water-soluble extractive values were 9.56% (w/v) and 16.48% (w/v) respectively. The percent extractives in various solvents indicate the quantity and nature of chemical constituents in the extracts. The extractive values are also useful in determination of specific chemical constituents soluble in a particular solvent. Extractive values of the plant with different solvents give a preliminary idea of the percentage (%) of the compounds extracted [22]. Determination of volatile content is a very important physical parameter for checking the quality and purity of aromatic plant. The volatile oil content was 1.2%w/w. The values obtained after the physical evaluation of the crude drug such as foreign matter, ash values, extractive values and volatile oil contents compare as standard data (pharmacopeial limits, [23] shown in Table 1 for the validation (identification, authentication and quality control) of the ginger rhizome.

Table 1: Physico-chemical properties of ginger fruits

S. No	Parameters	Observation Values (%)	Pharmacopeia limits
1	Foreign matter (% w/w)	0	NMT 1%
2	Extractive values a. Alcohol (% w/w) b. Water (% w/w)	 9.56 ± 1.02 16.48 ± 2.09	 NLT 3% NLT 10%
3	Ash Values a. Total ash (% w/w) b. Acid insoluble ash (% w/w)	 5.44 ± 0.28 0.5 ± 0.01	 NMT 6% NMT 1.5 %
4	Volatile oil content (% v/w)	1.2 ± 0.03	-

3.3 Chemo-profile of ginger oil

Ginger oil is extracted from *Z. officinale* rhizomes, which its chemical composition influences from geographical region and climatic conditions. 53 components were identified. Camphene (18.28%), para-cineole (10.02%), zingiberene (6.12%), and cuminaldehyde (2.17%) were major components in the oil of ginger (It is shown in table 2, Figure 2and 3). All these volatile components are biologically very active and responsible for various pharmacological activities. The antimicrobial, analgesic, anti-inflammatory, anti-ulcer, antioxidant, immunomodulatory, relaxant, antidiabetic, hypolipidaemic, anticancer, anti-oxidative stress, and warming effects of ginger oil or ginger distillate have been confirmed in experimental and preclinical studies [6-15]. The safety profiles of ginger oils are well documented and are generally regarded as safe. Due to wide pharmacological effects of ginger oil or ginger distillate, attention to ginger oil or ginger distillate as an ingredient of natural formulations in management of gastrointestinal and respiratory diseases is valuable. All major chemical components are shown in figure 2.

Table 2: Results of chemical compositions of ginger oil

S. No	Compounds	KI	Area%
1	Tricyclene	926	0.2842
2	α-Pinene	943	3.45
3	Camphene	954	18.2806
4	2-Heptanol	957	0.2337
5	β-Myrcene	986	1.5606
6	6-Meyhyl Hept-5-en-2-one	994	0.2337
7	α-Phellandrene	1000	0.0294
8	2-Carene	1003	0.471
9	α-Terpinene	1008	1.9705
10	Cymene	1020	2.1129
11	Para-Cineole	1027	10.0203
12	D-Limonene	1030	2.671
13	γ-Terpinene	1057	2.6023
14	Trans-linalool oxide	1081	0.2337
15	Linalool	1089	2.1777
16	2-Nonanol	1092	0.471
17	Undecanone	1100	1.2401
18	β-Citronellol	1117	2.0897
19	Camphor	1133	0.2337
20	Sabinol	1137	0.544
21	Isoborneol	1154	1.4095
22	Borneol	1164	0.7609
23	4-Terpineol	1174	0.4209
24	Myrtenal	1196	0.1575
25	Nerol	1218	0.7609
26	Cuminaldehyde	1239	2.1709

27	Geraniol	1240	0.9137
28	Linyl acetate	1244	1.1177
29	Borneol acetate	1268	3.3269
30	2- undecanone	1276	0.0554
31	Undecanone	1284	0.229
32	Thymol	1295	0.5651
33	Neric acid	1316	0.246
34	Neryl acetate	1342	0.3234
35	Geranic acid	1362	0.2939
36	Geraniol acetate	1366	0.9808
37	Farnesene	1447	1.7088
38	Thujopsene	1451	0.7314
39	Guaiol	1456	0.3168
40	Germacrene	1479	0.3248
41	Zingiberene	1487	6.1175
42	β-Sesquiphellandrene	1515	3.2254
43	Cadinene	1523	0.2973
44	Trans-Nerolidol	1526	0.246
45	Elemol	1540	0.5651
46	Elemol	1540	0.7188
47	Eudesm-4(14)-en-11-ol	1542	1.3141
48	Cubenol	1560	0.2296
49	Zingerone	1625	0.3008
50	β-Bisabolol	1650	0.5875
51	Farnesene epioxide	1763	0.4853
52	Curcumenyl acetate	1801	0.1378
53	Cinnamyl cinnamate	2057	0.4096

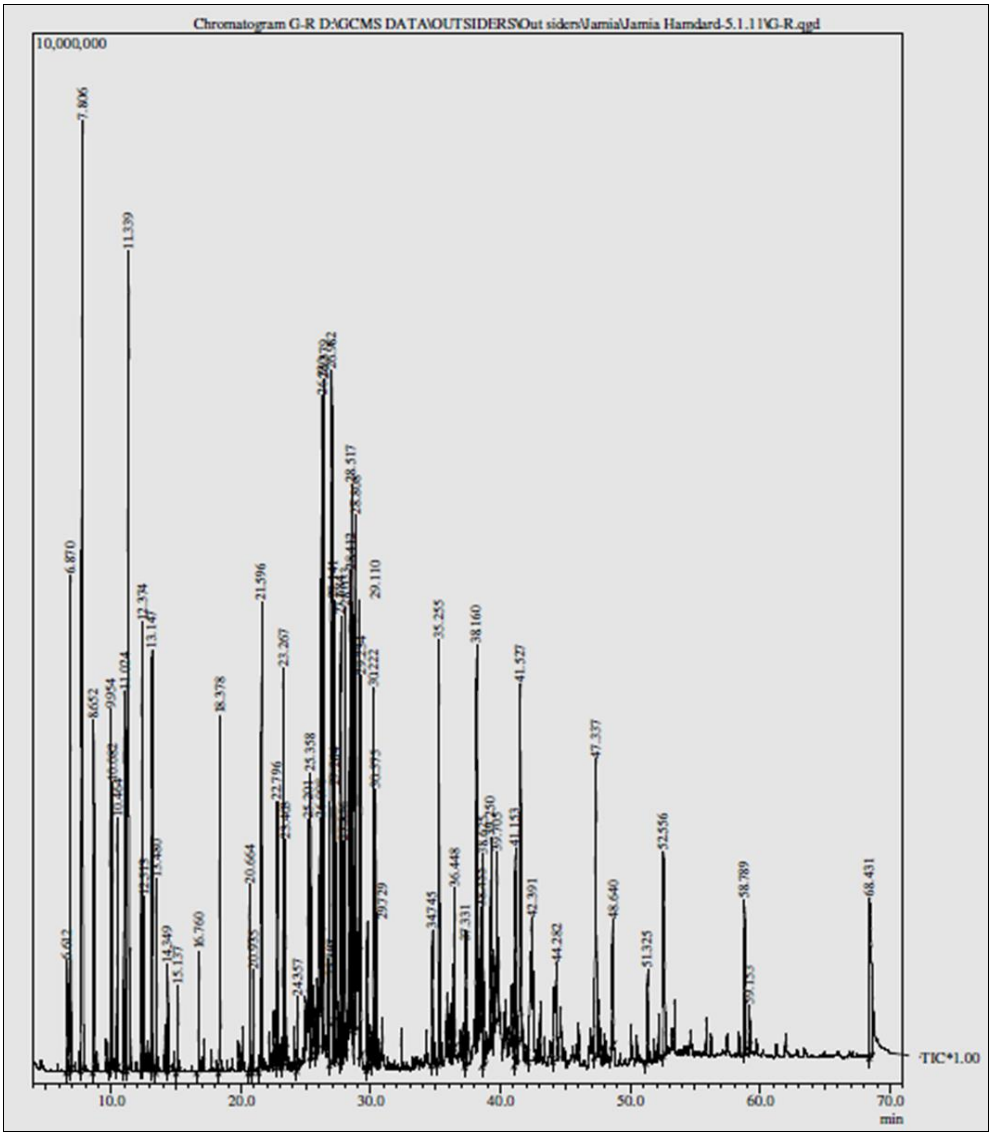


Fig 2: GC-MS chromatogram

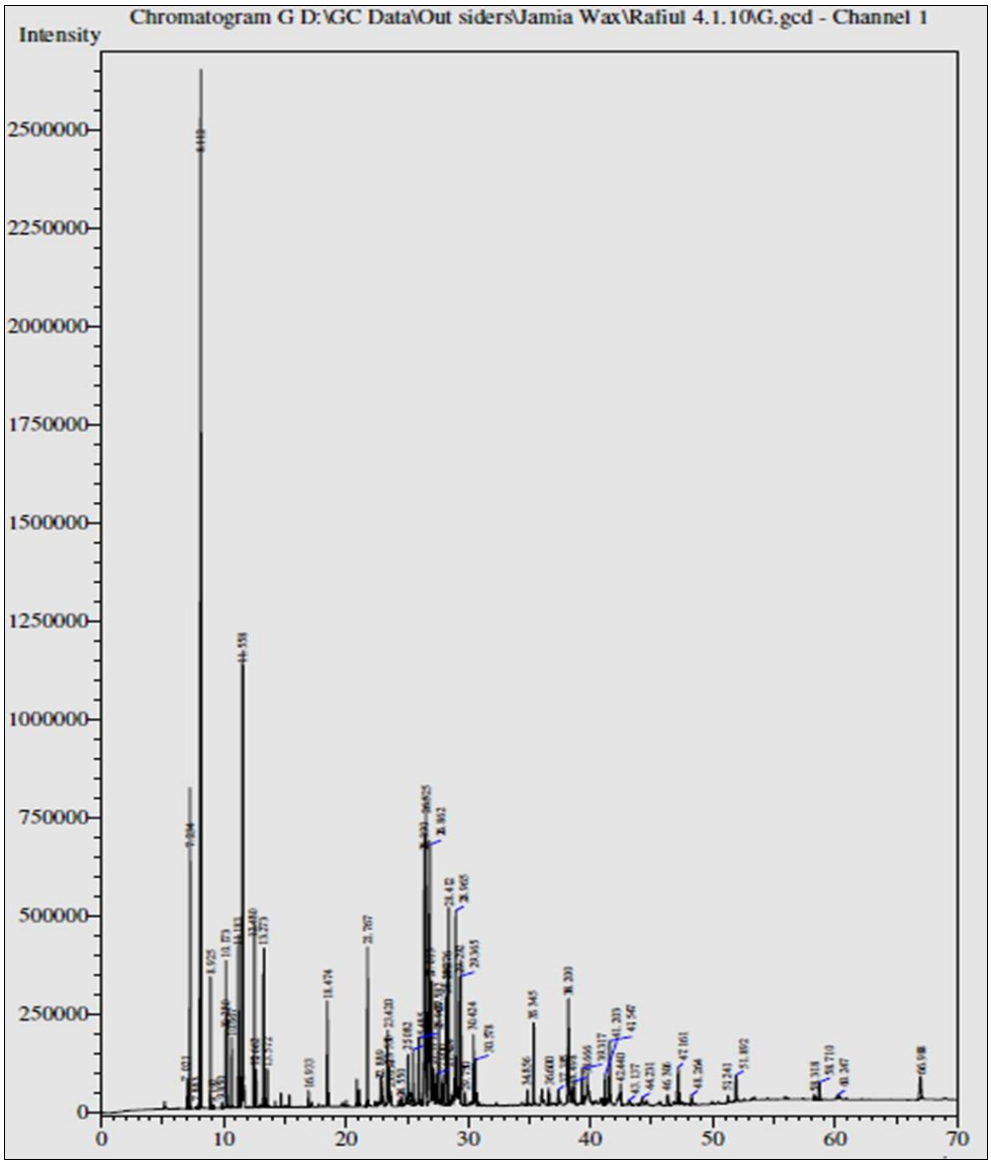


Fig 3: GC- chromatogram

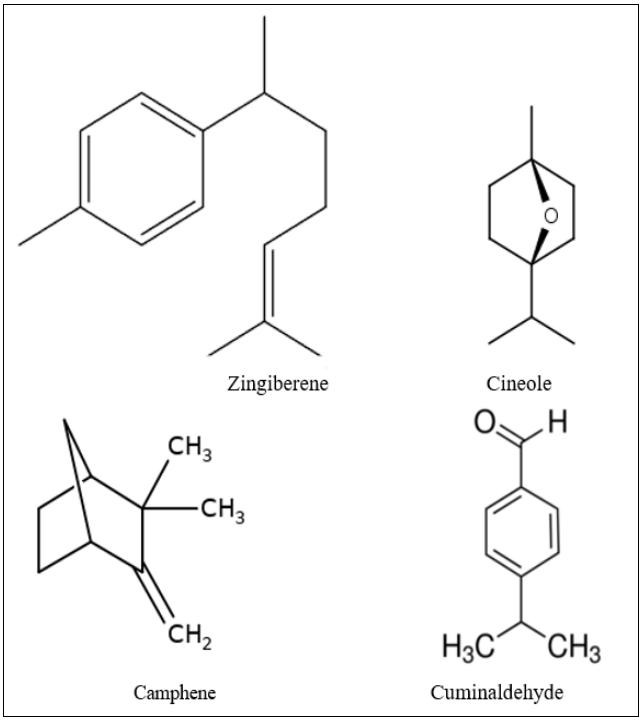


Fig 4: Structure of major chemical components in ginger oil

3.4 Toxic Chemical analysis

Aflatoxins were classified as B (blue fluorescence) and G (green fluorescence). As some plant secondary metabolites produced by moulds could be adverse affect to humans, the European legislation has set maximum levels of mycotoxins for a variety of spices. Microbiological quality evaluation of spices or crude drug should include mycotoxin contamination, especially of the parts at higher risk of contamination or spices from hot and humid climates [18]. Aflatoxin was absent in the ginger rhizome (Table 3). Thus the drug may be protected from acute toxicity and liver carcinogenicity due to lack of aflatoxins toxicity. Pesticides are organic compounds used to control or eliminate pests and the main unwanted effects associated with overexposure to pesticides are symptoms of the nervous system, including headache, dizziness, paraesthesia, tremor, discoordination, or convulsions. Pesticide residue such as o,p-DDD, p,p'- DDD, o,p-DDE, p,p'-DDE, o,p'-DDT, p,p'-DDT, Endosulfan, α-HCH, β-HCH, γ- HCH, δ-HCH etc was absent in the drug (Table 4). Toxic elements are extensively distributed throughout nature and occur freely in the environment. As they are likely to be present in many spices, it is important to reduce the total population exposure to toxic elements by minimizing contamination of herbal products. Heavy metal

like arsenic, lead, cadmium and mercury were not detected in the drug (Table 5). These results indicated that marketed ginger had good quality and it can be used in preparation of polyherbal formulation easily.

Table 3: Observations of aflatoxins residues

S. No.	Test Parameter	Test Method	Result
1.	Aflatoxin B1	AOAC 990.33	Not Detected
2.	Aflatoxin B2	AOAC 990.33	Not Detected
3.	Aflatoxin G1	AOAC 990.33	Not Detected
4.	Aflatoxin G2	AOAC 990.33	Not Detected

Table 4: Observations of pesticides residues

S. No.	Pesticides	Test Method	Result
1	α-BHC	AOAC 970.52/EPA 525.2	Not Detected
2	β-BHC	AOAC 970.52/EPA 525.2	Not Detected
3	γ-BHC(Lindane)	AOAC 970.52/EPA 525.2	Not Detected
4	δ-BHC	AOAC 970.52/EPA 525.2	Not Detected
5	Heptachlor	AOAC 970.52/EPA 525.2	Not Detected
6	Heptachlor_Epoxide	AOAC 970.52/EPA 525.2	Not Detected
7	α-Chlordane	AOAC 970.52/EPA 525.2	Not Detected
8	α-Endoulfan	AOAC 970.52/EPA 525.2	Not Detected
9	β-Chlordance	AOAC 970.52/EPA 525.2	Not Detected
10	Endrin	AOAC 970.52/EPA 525.2	Not Detected
11	Total DDE	AOAC 970.52/EPA 525.2	Not Detected
12	Total DDD	AOAC 970.52/EPA 525.2	Not Detected
13	Total DDT	AOAC 970.52/EPA 525.2	Not Detected
14	β-Endoulfan	AOAC 970.52/EPA 525.2	Not Detected
15	Endrin _Aldehyde	AOAC 970.52/EPA 525.2	Not Detected
16	Endoulfan_sulfate	AOAC 970.52/EPA 525.2	Not Detected
17	Aldrin	AOAC 970.52/EPA 525.2	Not Detected
18	Endrin_Ketone	AOAC 970.52/EPA 525.2	Not Detected
19	Methoxychlor	AOAC 970.52/EPA 525.2	Not Detected
20	Dieldrin	AOAC 970.52/EPA 525.2	Not Detected
21	Alachlor	AOAC 970.52/EPA 525.2	Not Detected
22	Butachlor	AOAC 970.52/EPA 525.2	Not Detected
23	Monocrotophos	AOAC 970.52/EPA 525.2	Not Detected
24	Phorate	AOAC 970.52/EPA 525.2	Not Detected
25	Mevinphos	AOAC 970.52/EPA 525.2	Not Detected
26	Dimethoate	AOAC 970.52/EPA 525.2	Not Detected
27	Malathion	AOAC 970.52/EPA 525.2	Not Detected
28	Methyl-parathion	AOAC 970.52/EPA 525.2	Not Detected
29	Chlorpyrifos	AOAC 970.52/EPA 525.2	Not Detected
30	Ethion	AOAC 970.52/EPA 525.2	Not Detected
32	Simazine	AOAC 970.52/EPA 525.2	Not Detected
33	Diazinon	AOAC 970.52/EPA 525.2	Not Detected
34	Phosphamidon	AOAC 970.52/EPA 525.2	Not Detected
35	Fenitrothion	AOAC 970.52/EPA 525.2	Not Detected
36	Fenthion	AOAC 970.52/EPA 525.2	Not Detected
37	Phosalone	AOAC 970.52/EPA 525.2	Not Detected
38	Quinalphos	AOAC 970.52/EPA 525.2	Not Detected
39	Coumaphos	AOAC 970.52/EPA 525.2	Not Detected
40	Parathion	AOAC 970.52/EPA 525.2	Not Detected
41	Malaoxon	AOAC 970.52/EPA 525.2	Not Detected
42	Dichlorvos	AOAC 970.52/EPA 525.2	Not Detected
43	2,4-D	PAM Vol I / EPA 515.3	Not Detected

Table 5: Observations of toxic metal residues

S. No.	Test Parameter	Test Method	Result
1.	Cadmium (Cd)	ICP-OES	Not Detected
2.	Lead (Pb)	ICP-OES	Not Detected
3.	Arsenic (As)	ICP-OES	Not Detected
4.	Mercury (Hg)	ICP-OES	Not Detected

3.5 lipid profile and pancreatic lipase studies

HFD-induced hyperlipidemia is well reported (Haque and Ansari, 2018). Our data also showed that rats in the HFD group exhibited significantly higher TG, TC and LDL-C levels, and lower HDL-C. However, the administration of ZD or orlistat significantly lowered the TG, TC and LDL-C levels and higher HDL-C in serum (Table 6). It is reported that obesity is associated with dyslipidemia, characterized by

elevated TG and reduced HDL-C concentrations. Pancreatic lipase is the main enzyme for fat absorption that hydrolysis triglycerides in the GI tract. Pancreatic lipase inhibitor which assists to limit intestinal fat absorption at the initial stage, have been proved as helpful medications for the treatment of hypolipidemic agents. ZD did not significantly inhibit the pancreatic lipase activity on obese rats. Thus ZD showed good hypolipidemic action without inhibition of pancreatic lipase activity.

Table 6: Effect of ZD on lipid levels

Groups	TG level (mg/dl)	TC level (mg/dl)	HDL-C level (mg/dl)	LDL-C (mg/dl)
Control	45.60 ± 2.51	60.80 ± 2.18	33.18 ± 0.89	19.86 ± 0.81
HFD	105.11 ± 4.37$^{\$\$}$	97.50 ± 2.88$^{\$\$}$	23.07 ± 2.59$^{\$\$}$	51.89 ± 3.81$^{\$\$}$
HFD+ ZD	42.20 ± 2.96**	72.89 ± 1.61**	27.55 ± 2.34**	18.31 ± 1.45**
HFD + O	42.20 ± 3.361**	72.08 ± 1.14**	27.66 ± 1.09**	32.9 ± 1.81**

All values were expressed as mean ± SEM for six rats in each group. $^{**}p<0.001$ as compared to with HFD group. $^{\$\$}$ $p<0.001$ as compared to control group

Table 7: Effect of ZD on serum pancreatic lipase activity

Groups	Pancreatic lipase activity (U/L)
Control	165.03 ± 13.55
HFD	559.18 ± 36.02$^{\$\$}$
HFD + ZD	550.38 ±5.54
HFD + orlistat	160.02 ± 7.18**

All values were expressed as mean ± SEM for six rats in each group. $^{**}P<0.001$ as compared with HFD group. $^{\$\$}P<0.001$ as compared with control group

4. Concluding remark

The different physicochemical parameters are also showing good results and found under pharmacopeial limits. These physical parameters will help to identify the authenticity of the drug after comparing with pharmacopeial limits. In the conclusion, it will serve as standard method for the evaluating quality control of the preparation containing plants and also can helpful in distinction of other allied species and adulterants. A phytochemical screening by GC and GCMS may be useful in the estimation of bioactive principle having therapeutic properties. This article may help all researchers, pharmacological investigators and dosage form formulators and pharmaceutical scientists to develop a new way of validation in the field of health science. Moreover, it can be concluded from the current study that, ZD ameliorate hyperlipidemia via hyplipidemic action without any pancreatic lipase inhibition. Thus ZD may be a tremendous alternative strategy for developing effective and safe hypolipidemic drugs in the future.

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