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Phytochemical profiling and antioxidant activity of *Zingiber officinale* rhizome

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Abstract

Diabetes mellitus is a serious public health problem that has a strong negative impact on the health-related quality of life. Finding a solution to such disease living in the Diabetic capital is possible by the use of bioactive compounds of medicinal plants which are produced during secondary metabolism. There are many plants, spices and herbs including the kitchen ingredients having the therapeutic properties. Especially ginger which contributes more in our routine diet, is possessing therapeutically effects on many ailments. Hence an effort has been made to analyze and quantify the bioactive compounds and assess the antioxidant capacity in various extracts of *Zingiber officinale* rhizome. The presence of alkaloids, flavonoids, phenols, tannins, terpenoids, saponins, phytosterols and anthroquinones in various extracts of *Zingiber officinale* rhizome was confirmed through qualitative test. The total antioxidant capacity of fresh and dehydrated samples of *Zingiber officinale* rhizome was 872.97 mg and 1251.882 mg AA/100g respectively. Seventy compounds were identified in the hexane extract of *Zingiber officinale* rhizome, out of which Gingerol, β -Bisabolol that have been found which are supposed to be the leading players for diabetes type 2 treating therapy and thereby ginger and their constituents will show pivotal role in the control of diabetes and its complications via anti-hyperglycemic effect.

Keywords: Ginger rhizome, phytochemical profiling, antioxidant activity, qualitative and quantitative test – GC-MS

Introduction

Medicinal herbs or plants are considered as a key ingredients which can be used in drug development either pharmacopoeial, non-pharmacopoeial or synthetic drug or culinary purpose. Plants are an important source of medicine and play a key role in human health (Sandberg and Corrigan, 2001) [22]. Phytochemicals are the bioactive chemicals of plant origin as secondary metabolites because the plants that manufacture them may have little need for them (Molyneux *et al.*, 2007) [17]. They are synthesized in all parts of the plant body such as leaves, stem, bark, root, flower, fruits, seeds, etc., i.e. any part of the plant body may contain active components. This chemicals work with nutrients and fibers to form an integrated parts of the defense system against various diseases and stress conditions (Edeoga, 2005) [6].

Medicinal plants with culinary purpose prove to be a great ingredient which everyone needs to incorporate in their daily foods. Historically, natural products have been used since ancient times and in folklore for the treatment of many diseases and illnesses. Ginger (*Zingiber officinale*) is used worldwide as a cooking spice, condiment and herbal remedy. It is common dietary spice also traditionally used in the treatment of various diseases including diabetes mellitus (Mbaveng and Kuete, 2017) [15].

Ginger is carminative, pungent, stimulant, used widely for indigestion, stomachache, malaria and fevers. It is mainly used to cure diseases due to morbidity of Kapha and Vata. Ginger with lime juice and rock salt increases appetite and stimulates the secretion of gastric juices. It is also used for abdominal pain, anorexia, arthritis, atonic dyspepsia, bleeding, cancer, chest congestion, chicken pox, cholera, chronic bronchitis, cold extremities, colic, colitis, common cold, cough, cystic fibrosis, diarrhoea, difficulty in breathing, dropsy, fever, flatulent, indigestion, disorders of gallbladder, hyperacidity, hypercholesterolemia, hyperglycemia, indigestion, morning sickness, nausea, rheumatism, sore throat, throat ache, stomach ache and vomiting (Moghaddasi and Kashani, 2012) [16].

The plant has a number of chemicals responsible for its medicinal properties and pharmacological applications *viz.*, anti-arthritis, anti-inflammatory, anti-diabetic, antibacterial, anti-fungal, anti-oxidant, anti-tumor, anti-cancer, anti-proliferative, anti-platelet activities etc. (Mbaveng and Kuete, 2017; Guk Shin *et al.*, 2005) [15, 23].

Extracts of ginger have pronounced antioxidant activity comparable to that of synthetic antioxidant preservatives (Moghaddasi and Kashani, 2012) [16]. A wide variety of chemical constituents have been isolated and identified from extracts of *Zingiber officinale*. The biological activity of the compounds have been studied and documented. Gingerols are the active components of ginger (Shoji, 1982; Kobayashi, 1988). Zingerone (4(4-hydroxy-3-methoxyphenyl)-2-butanone) is a nontoxic and inexpensive compound with varied pharmacological activities and it is the least pungent component of *Zingiber officinale*. Zingerone is absent in fresh ginger but cooking or heating transforms gingerol to zingerone. Zingerone has potent anti-inflammatory, antidiabetic, antilipolytic, antidiarrhoeic, antispasmodic, and so forth properties. Besides, it displays the property of enhancing growth and immune stimulation. It behaves as appetite stimulant, anxiolytic, antithrombotic, radiation protective, and antimicrobial. Also, it inhibits the reactive nitrogen species which are important in causing Alzheimer's disease and many other disorders (Ahmad *et al.*, 2015) [1]. Many studies stated that nutraceutical effects of ginger against lifestyle disorders like cardiovascular diseases, diabetes mellitus, and cancer. Likewise, the study was planned to assess the presence of bioactive compounds through qualitative and quantitative measurements; assess the

antioxidant capacity and to identify the phytochemicals in the ginger by GC-MS.

Methods

i. Preliminary screening of Phytochemicals

Solvent extraction (Maceration): The samples were extracted by solvent extraction method especially maceration method (24 hr soaking). The solvents used for this study are ethanol, hexane, petroleum ether and aqueous medium. The powdered sample (25 g) was soaked in 250 ml of each solvent at room temperature and it was kept in rotary shaker at 100 rpm for 24 hrs. It was filtered rapidly taking precautions against loss of solvents, initially it was filtered with muslin cloth and then it was filtered through Whatman No.1 filter paper and the filtrate obtained was evaporated / concentrated by using Flash Evaporator and then it was further used for the bioactive components screening by qualitative test.

Qualitative analysis of phytochemicals

All the extracts *viz.*, petroleum ether, hexane, ethanol, and aqueous extracts of *Zingiber officinale* rhizome were subjected to qualitative phytochemical tests (table 1) for different constituents such as alkaloids, terpenoids, anthroquinones, flavonoids, tannins, phenols, saponins, phytosterols, carbohydrates and proteins.

Table 1: Qualitative test for phytochemical screening

S. No.	Bioactive Components	Test	End point
1.	Alkaloids	Marquis Reagent	Formation of dark orange or purple colour
2.	Saponin	Foam or Froth	Formation of foam stable for 10 min
3.	Phenols	Ferric chloride	Formation of blue, black or violet colour
4.	Tannins	Braymers	Formation of blue colour
5.	Flavonoids	Shinoda	Colour change from yellow to colourless
6.	Phytosterols	Libermann-Burchard's	Array of colour change
7.	Terpenoids	Salkowski	Formation of reddish brown
8.	Anthroquinones	Borntrager's test	Formation of pink red colour
9.	Carbohydrates	-	Formation of red or dull violet
10.	Proteins	-	Formation of violet colour

1. Detection of Alkaloids

To 1 ml of each extract, 1 ml of Marquis Reagent, 2 ml of concentrated sulphuric acid was added and few drops of 40% formaldehyde was added and mixed, appearance of dark orange or purple color indicates the presence of alkaloids.

2. Detection of Saponins

Foam Test: A small quantity of the extract was diluted with distilled water to 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. A two centimeter layer of foam or froth which is stable for 10 minutes indicates the presence of saponins.

3. Detection of Phenols

Ferric chloride test: About 50 mg of extract was dissolved in distilled water and to this few drops of neutral 5% ferric chloride solution was added. Formation of blue, green, black and violet color indicates the presence of phenols.

4. Detection of Tannins

Folin Dannis reagent (0.5ml) was added to the extract and then maintained for 5min at room temperature. Then, 2ml of 20% sodium carbonate was added and left for 5 minutes. Formation of blue color indicates the presence of tannins.

5. Detection of Flavonoids

Alkaline Reagent Test: Addition of 5 drops of 5% Sodium hydroxide to 1 ml of the test solution resulted an increase in the intensity of the yellow color which became colorless on addition of a few drops of 2 M hydrochloric acid which indicated the presence of flavonoids.

6. Detection of Phytosterols

Libermann-Burchard's: The extract 0.05 ml is dissolved in 2ml of acetic anhydride. To this 1 or 2 drops of concentrated sulphuric acid was added along the sides of the tube. An array of color change shows the presence of phytosterols.

7. Detection of Terpenoids

Salkowski test: The extract was dissolved in 2ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the phytosterols and sterols compound, in the extract.

8. Detection of Anthroquinones

To 2ml of extract, 1 ml of dilute 10% ammonia and few drops of chloroform was added and shaken well. A pink red color in lower layer shows anthracene derivatives.

9. Detection of Carbohydrates

Take 1ml of extract, add few drops of Molisch's reagent and then add 1ml of concentrated H₂SO₄ at the side of the tubes. The mixture was then allowed for 2 to 3 minutes. Formation of red or dull violet color indicates the presence of carbohydrates.

10. Detection of Proteins

2ml of each extract, 1ml of 40% sodium hydroxide and few drops of 1% copper sulphate were added. Formation of violet colour indicates the presence of proteins.

ii. Quantification of Total Phenol, Flavonoid content and Total Antioxidant Capacity

Sample preparation

Known quantities of fresh and dried samples (2 - 5g) were taken and 15ml of 80% methanol (pH 2.0) was added and kept at room temperature for 30 minutes. The three supernatants were pooled, centrifuged at 6000 rpm for 15 min and filtered through Whatman No.1 filter paper. Made up the volume to 50 ml with the solvent and transferred the sample to micro centrifuge tubes and stored at -18°C for TPC, TFC and TAC determination.

1. Total phenol

The determination of total phenol based on Folin-Ciocalteu reagent assay (Singleton and Rossi, 1965). For this method of estimation, Gallic acid (100 mg%) was used as a standard solution. The TPC of fresh and dried samples expressed as mg gallic acid equivalents (GAE)/100 g. Taken known aliquot of sample and made volume up to 1.5 ml with D/W, then 0.5 ml of folin-ciocalteu reagent was added. Ten ml of 7.5% Na₂CO₃ added and incubated at 37°C for 60 mins. Read the resulting blue colour complex at 750nm.

$$\text{Total phenol (mg GAE/100g)} = \frac{\text{Std. Conc} \times \text{Sample OD} \times \frac{\text{Vol. made up}}{1000}}{\text{Std. OD} \times \frac{\text{Aliquot Wt. of sample}}{1000}} \times 100$$

2. Total flavonoid: Total Flavonoid content was measured by the aluminium chloride colorimetric assay (Zhisten *et al.*, 1999) [30]. Rutin (10 mg %) was used as a standard. The TFC of fresh and dried samples expressed as mg Rutin equivalents (RE) /100 g. Taken known aliquot 2 ml of sample and made volume up to 5 ml with D/W. Added 0.3 ml of 5% NaNO₂. After five minutes, 0.6 ml of 10% (hydrated form) AlCl₃ was added and mixed. After six minutes, added 2ml of 1N NaOH and then mixed thoroughly. Then 2.1 ml D/W was added to make volume up to 10 ml. The absorbance of resulting pink colour is read at 510 nm against blank.

$$\text{Total flavonoid (mg RE /100g)} = \frac{\text{Std. Conc} \times \text{Sample OD} \times \frac{\text{Vol. made up}}{1000}}{\text{Std. OD} \times \frac{\text{Aliquot Wt. of sample}}{1000}} \times 100$$

3. Total Antioxidant Capacity

Procedure for Total Antioxidant Capacity by DPPH RSA

Total antioxidant capacity was determined by DPPH assay (2, 2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity (Brand-Williams *et al.*, 1995) [4]. Sample (0.3, 0.5, 0.7ml) taken and made up to 1ml with methanol, Standard (0.1,0.2,0.3,0.4 ml) was taken and made up to 1ml with methanol, Blank (1ml methanol) were taken. Three ml of DPPH reagent was added and incubated for 20 min at 37°C and absorbance was read at 517 nm. L-ascorbic acid was used as a standard in the concentration of 10 mg%. Percent inhibition and total antioxidant capacity (mg ascorbic acid/100 g) were measured.

$$\% \text{ inhibition of the Sample} = \frac{\text{Control (OD)} - \text{Sample (OD)}}{\text{Control (OD)}} \times 100$$

$$\% \text{ inhibition of the Standard} = \frac{\text{Control (OD)} - \text{Standard (OD)}}{\text{Control (OD)}} \times 100$$

$$\text{TAC (mg AAE/100g)} = \frac{\text{Std. conc.}}{\text{Std \% inhibition}} \times \frac{\text{Sample \% inhibition}}{\text{Aliquot taken}} \times \frac{\text{Vol made up}}{\text{Sample taken}} \times \frac{100}{1000}$$

iii. Identification of phytochemicals by GC-MS

Sample preparation

The plant was cleaned and dried in cabinet drier at 40°C and it was finely powdered. The powdered sample was used for GC-MS analysis. 10g of powder was taken and saturated in 100ml of HPLC graded solvent like hexane. It was left for 24 hrs by frequent shaking of sample. It was initially filtered with muslin cloth and then with Whatman No.1 filter paper. The filtered extract was then concentrated in Flash Evaporator after which it was filtered with anhydrous sodium sulphate to get water free extract. The water free extract was used for analysing bioactive components by GC-MS.

Procedure for GC-MS: GC-MS analysis was carried out on Shimadzu GC-MS QP 2020 system comprising auto sampler

and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument employing following conditions: Column Elite-1 fused silica capillary column (30mm×0.25mm I.D ×1 μ M df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 1.0μl was employed (split less) injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min, to 200 °C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time is 34 minutes.

Statistical analysis

All measurements were performed in triplicate for the samples. The results were pooled and expressed as Mean and Standard Deviation.

Result and Discussion

i. Preliminary screening of Phytochemicals (Qualitative test):

Qualitative test for screening of bioactive components such as alkaloids, flavonoids, phenols, tannins, terpenoids, saponins, phytosterols, anthraquinones, carbohydrate and protein were done in ethanol, hexane, petroleum ether and aqueous medium of extracts of *Zingiber officinale* rhizome by adopting standard methods. Table 2 shows qualitative phytochemical composition of various extracts of *Zingiber officinale* rhizome. The results of the bioactive compounds qualitative tests in *Zingiber officinale* rhizome confirmed that the presence of alkaloids, flavonoids, phenols, tannins, terpenoids, phytosterols and carbohydrate in all the extracts. A test of saponins showed the presence in hexane and petroleum ether extracts. It was also revealed that the presence of anthraquinones in ethanol and aqueous extracts; and the presence of protein in of ethanol and petroleum ether. All tested compounds were found present in one solvent extract or the other. Among the solvents, all three viz., ethanol, hexane and petroleum ether has the potential presence of various bioactive components than the aqueous medium.

Pius *et al.* (2015) [21] reported that phytochemical analysis of the ginger extracts and indicated that the methanolic extract possessed phenolics, saponin, tannin and flavonoids, but no glycoside detected. On the other hand only cardiac glycoside, out of the 5 phytochemicals, was detected from the aqueous extract of ginger. Compounds present in ginger have shown to reduce the severity of several diseases especially in the GI tract and the studies have clearly indicated that the various extracts of ginger and its principal phytochemicals – gingerols, zingerone, shogaols, and paradols – have a protective role in gastric ailments and irritations such ulcers, vomiting, nausea, dyspepsia, stomach ache, spasm, and gastrointestinal cancer (Kaur *et al.*, 2015) [9]. Ghosh *et al.* (2011) [7] reported that ginger oil is a mixture of constituents which consisting of monoterpenes (phellandrene, camphene, cineole, citral, and borneol) and sesquiterpenes (zingiberene, zingiberol, zingiberenol, β -bisabolene, sesquiphellandrene, and others) and also present the aldehydes and alcohols [Tang and Eisenbrand (1992) [26] and Suekawa *et al.*, (1984) [25].

Table 2: Bioactive components of various extracts of *Zingiber officinale* rhizome

Metabolites	Ethanol	Hexane	Petroleum ether	Aqueous medium
Alkaloids	++	++	+	+
Saponins	-	+	+	-
Phenols	+	++	++	+
Tannins	++	++	+	+
Flavonoids	+	++	++	+
Phytosterols	+	+	+	+
Terpenoids	+	++	++	+
Anthroquinones	+	-	-	++
Carbohydrate	++	++	++	+
Protein	+	-	+	-

Note: +++ (Much Abundant), ++ (Medium), + (Less), - (Absent)

ii. Quantitative analysis of phytochemical in *Zingiber officinale* rhizome

The Total Phenol Content (TPC) and Total Flavonoid Content (TFC) of extracts of fresh and dehydrated (by cabinet drier at 40°C) *Zingiber officinale* rhizome were quantified. The free radical scavenging potential of fresh and dried rhizome of *Zingiber officinale* was tested by the DPPH. The results are shown in table 3. The total antioxidant capacities of fresh and dehydrated samples of *Zingiber officinale* rhizome were 872.97 mg and 1251.882 mg AA/100g respectively. Gulcin *et al.* (2016) stated that the antioxidant activity of dried ginger essential oil was 83.87% and fresh ginger essential oil 83.03% with an allowance of 0.50. The total phenol content of fresh and dehydrated samples of *Zingiber officinale* were 52.229 mg GAE/100gm and 99.153 mg GAE/100 gm respectively. Ali *et al.* (2018) [2] stated that the total phenolic content in ginger rhizome was 60.34 ± 0.43 mg gallic acid/g. The total flavonoid content of fresh and dehydrated samples of *Zingiber officinale* rhizome were 219.899 mg RE/100gm and 398.008 mg RE/100gm respectively. Ali *et al.* (2018) [2] stated that the total flavonoid content in ginger rhizome 40.25 ± 0.21 mg quercetin/g. Among the samples, the dehydrated samples had higher amount of TPC, TFC and TAC than the fresh samples of both. Ghosh *et al.* (2011) [7] reported that ginger roots' extracts contain polyphenol compounds ([6]-gingerol and its derivatives), which have a high antioxidant activity. Studies in animal models have shown that ginger and its phenolic constituents suppress carcinogenesis in the skin (Park *et al.*, 1998; Murakami *et al.*, 2004) [20, 18], gastrointestinal tract (Yoshimi *et al.*, 1992) [28], colon (Bode, 2003; Manju and Nalini, 2005) [3, 14], and breast (Nagasawa, *et al.*, 2002) [19].

Table 3: Quantitative analysis of phytochemical in *Zingiber officinale* rhizome

Samples	TP (mg GAE/ 100g)	TFC (mg RE/ 100g)	Antioxidant activity (DPPH Assay)	
			% Inhibition	TAC (mg AA/100g)
<i>Zingiber officinale</i> rhizome (Fresh)	52.23	219.90	52.85	872.98
<i>Zingiber officinale</i> rhizome (Powder)	99.15	398.01	75.79	1251.88

iii. Identification of phytochemicals in *Zingiber officinale* Rhizome by GC-MS: Gas Chromatography-Mass Spectrometric spectrum of the hexane extract of *Zingiber officinale* rhizome showed 70 major peaks (Fig 1.) indicating

that the presence of seventy bio-active compounds in the extract. Chemical compounds identified with their name, area, retention time and% of peak area were presented in the Table 4.

Table 4: Identified phytochemical compounds in hexane extract of *Zingiber officinale* rhizome

Peak#	R. Time	Area%	A/H	Name of the compound
1	3.015	0.94	0.64	Camphene
2	3.066	0.10	1.64	5-Hepten-2-one,6-methyl-
3	3.119	0.32	1.89	.beta.-Myrcene
4	3.348	0.11	2.06	Bicyclo[3.1.0]hex-2-ene,2-methyl-5-(1-methyl-
5	3.599	3.91	2.98	.beta.-Phellandrene
6	4.210	0.41	3.19	Linalool
7	5.375	0.15	2.81	2-((3,3-Dimethyloxiran-2-yl)methyl)-3-methyl
8	5.667	0.56	2.73	Bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethyl-,(1
9	6.042	0.30	2.63	.alpha.-Terpineol
10	6.511	0.25	2.61	Citronellol
11	8.110	0.21	2.79	2-Undecanone
12	10.478	0.17	2.71	1,2,4-Metheno-1H-indene,octahydro-1,7a-dim
13	10.596	0.31	2.63	Copaene
14	10.902	0.29	2.85	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methyl-
15	12.426	0.29	2.92	(E)-.beta.-Farnesene
16	12.535	0.21	3.22	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methyl-
17	12.882	0.26	2.79	Alloaromadendrene
18	13.180	0.16	3.13	Naphthalene,decahydro-4a-methyl-1-methyl-
19	13.265	2.64	2.92	Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-
20	13.414	1.51	2.84	.beta.-copaene
21	13.664	19.19	3.52	(1S,5S)-2-Methyl-5-((R)-6-methylhept-5-en-2-yl)-
22	13.825	7.76	3.29	.alpha.-Farnesene
23	13.925	2.37	2.79	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-
24	13.990	3.59	2.58	.beta.-Bisabolene
25	14.207	0.45	2.72	cis-Muurolo-4(15),5-diene
26	14.293	0.26	4.50	(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyl-
27	14.422	7.73	2.96	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methyl-
28	14.496	0.19	2.30	(E)-1-Methyl-4-(6-methylhept-5-en-2-ylidene)-
29	15.279	0.38	2.73	1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-,(E)-
30	15.446	0.39	2.97	1,5-Cyclodecadiene,1,5-dimethyl-8-(1-methyl-
31	16.080	0.64	3.59	Bromoaceticacid,tetradecyl ester
32	16.697	0.51	2.82	(1R,4R)-1-methyl-4-(6-Methylhept-5-en-2-yl)-
33	17.108	0.38	3.69	(1R,4R)-1-methyl-4-(6-Methylhept-5-en-2-yl)-
34	17.224	0.19	2.82	(1aR,3aS,7S,7aS,7bR)-1,1,3a,7-Tetramethyldeca-
35	17.817	0.30	3.28	2-Naphthalenemethanol,decahydro-.alpha.,.al-
36	18.454	0.52	3.24	Cyclohexanol,3-ethenyl-3-methyl-2-(1-methyl-
37	18.675	0.20	3.67	6,10-Dodecadien-1-yn-3-ol,3,7,11-trimethyl-
38	18.783	0.20	3.02	Cyclohexanol,3-ethenyl-3-methyl-2-(1-methyl-
39	19.230	0.45	2.74	Methyltetradecanoate
40	20.865	0.48	2.70	1-Nonadecene
41	21.024	0.12	2.31	Octadecane
42	21.268	0.28	2.99	2-Butanone,4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-
43	23.248	1.93	2.73	9-Hexadecenoicacid,methylester,(Z)-
44	23.721	8.71	2.96	Hexadecanoicacid,methylester
45	23.820	0.18	3.41	ButylatedHydroxytoluene,TMSderivative
46	24.330	0.25	3.56	1,2-Benzenedicarboxylicacid,butyl2-ethylhe-
47	24.450	0.17	2.84	n-Hexadecanoicacid
48	24.898	0.17	2.71	(E)-1-(6,10-Dimethylundec-5-en-2-yl)-4-methyl-
49	25.161	0.41	2.60	1-Nonadecene
50	25.291	0.18	2.81	2-Methyltetracosane
51	25.921	0.23	2.95	1,6,10,14-Hexadecatetraen-3-ol,3,7,11,15-tetra-
52	27.105	3.35	2.87	9,12-Octadecadienoicacid(Z,Z)-,methylester
53	27.255	8.75	3.08	9-Octadecenoicacid,methylester,(E)-
54	27.346	0.72	2.82	11-Octadecenoicacid,methylester,(Z)-
55	27.431	0.40	3.01	6-Methyl-4,6-bis(4-methylpent-3-en-1-yl)cycl-
56	27.743	1.83	2.76	Methylstearate
57	28.265	0.21	2.89	1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10,
58	29.054	0.32	2.74	n-Tetracosanol-1
59	29.722	0.60	3.12	3-Decanone,1-(4-hydroxy-3-methoxyphenyl)-
60	30.834	2.41	3.40	1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-ol-
61	31.486	0.20	3.53	1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-di-
62	32.573	5.13	4.57	5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)de-
63	32.852	0.18	3.41	1-(3,4-Dimethoxyphenyl)-5-hydroxydecane-3-ol-
64	34.467	1.28	5.30	(3R,5S)-1-(4-Hydroxy-3-methoxyphenyl)deca-
65	34.805	0.32	2.98	1-(3,4-Dimethoxyphenyl)decane-3,5-diylidiac-
66	35.902	0.28	3.55	9-Hexacosene

67	36.063	0.18	3.31	5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)do
68	37.806	1.00	3.88	1-(4-Hydroxy-3-methoxyphenyl)tetradec-4-en
69	38.473	0.43	4.50	1-(4-Hydroxy-3-methoxyphenyl)tetradecane-3
70	45.646	0.56	5.64	(E)-4-(2-(2-(2,6-Dimethylhepta-1,5-dien-1-yl)
		100.00		

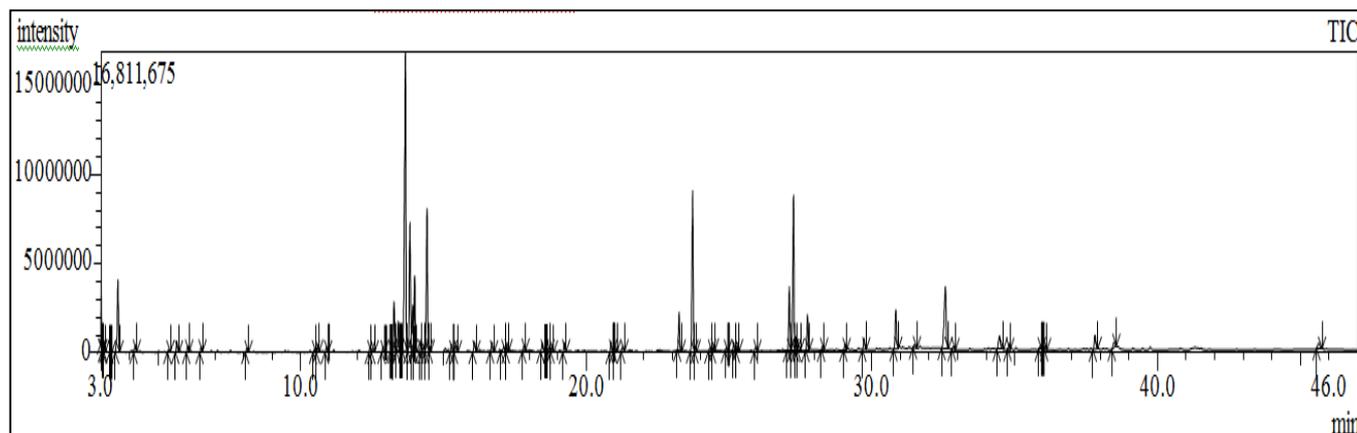


Fig 1: GC-MS Chromatogram of hexane extract of *Zingiber officinale* rhizome

It was found that the 15 main constituents are present in the hexane extract of *Zingiber officinale* rhizome. They are beta-phellandrene (3.91%), aphythalene,1,2,3,4,4a,5,6,8a-octahydro-7-m (2.79%), Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-m (2.96%), 9-Hexadecenoicacid methylester(Z)-(2.73%), alpha.-Farnesene (3.29%), 9,12-Octadecadienoicacid(Z,Z)- methylester (2.87%), 9-Octadecenoicacid (E)-methylester (3.08%), Methylstearate (2.76%), 1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-o (3.40%), beta-Bisabolene (2.58%), (3R,5S)-1-(4-Hydroxy-3-methoxyphenyl)deca (5.30%), 5-Hydroxy-1-(4-hydroxy-methoxyphenyl)de (4.57%) and (1S,5S)-2-Methyl-5-((R)-6-methylhept-5-en-2 (3.52%), Benzene 1-(1,5-dimethyl-4-hexenyl)-4-methyl (2.92%), beta.-copaene (2.84%). Ly-le (2014) studied those anti-diabetic activities of bioactive compounds in *Zingiber officinale*. The result showed that *Zingiber officinale* can be claimed that rhizome containing a variety of bioactive compounds namely 4-gingerol, 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol and β -Bisabolol that have been functional effects for diabetes type 2 treating therapy.

Krishnamoorthy *et al* (2014) ^[10] stated that the linoleic acid esters present in the stem, Hexadecanoic acid methyl ester, are reported to have anti-inflammatory, cancer preventive, hepatoprotective, antiarthritic, and anticoronary properties. Krishnamoorthy *et al* (2014) ^[10] also stated that 9-octadecenoic acid (Z)-, methyl ester, is having anti-inflammatory, antiandrogenic, and anemiagenic properties. Kalaivani *et al* (2013) ^[8] stated that methyl stearate is reported to have antidiarrheal and cytotoxic and antiproliferative property. Ahmad *et al* (2015) ^[1] stated that Zinger one has potent anti-inflammatory, antidiabetic, antilipolytic, antidiarrhoeic, antispasmodic, and so forth properties. Dai *et al.* (2013) ^[5] and Krist *et al.* (2015) ^[11] stated that beta-phellandrene is one of the constituent that contributes to the antimicrobial activity of the oil of the flower buds of *Wedelia trilobata*. Yeo *et al.* (2016) ^[27] stated that β -bisabolene, a sesquiterpene constituting 5% of the essential oil, exhibited selective cytotoxic activity for mouse and human breast cancer cells. Lana *et al* (2017) ^[12] stated that 9, 12-Octadecadienoic acid is the most abundant polyunsaturated fatty acid in human nutrition and it also plays an important

medicine for treatment of hyperlipidemia and atherosclerosis.

Conclusion

The dried powder of rhizome of *Zingiber officinale* was extracted with solvent extraction using various solvents such as petroleum ether, hexane, ethanol, and aqueous. It indicates that the presence of different phytochemicals in the *Zingiber officinale* extracts that confirms the ability to serve a source of natural medicines. The results of free radical scavenging potential of fresh and dried rhizome of *Zingiber officinale* showed that dehydrated samples had higher amount of TPC, TFC and TAC than the fresh samples of both. Seventy compounds were identified in *Zingiber officinale* extract by GC-MS analysis. *Zingiber officinale* containing a variety of bioactive compounds namely Gingerol, β -Bisabolol and various compounds that have been the leading players for diabetes type 2 treating therapy and thereby ginger and their constituents will show pivotal role in the control of diabetes and its complications via anti hyperglycemic effect. So the bioactive compound rich ginger can be consumed on the daily basis and it will enhance the anti-diabetic activities.

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