Extraction and Identification of Essential Oil by GCMS Analysis and Antioxidant Properties of Ginger

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Abstract

Firstly, a semi-quantitative elemental analysis of ginger was carried out by EDXRF method. From the analysis, potassium and calcium were observed as the major trace elements. Potassium is very important essential element for human metabolisms. It helps to reduce the hypertension. Calcium is effective for teeth and bone. The extraction of essential oil from ginger was carried out by steam distillation method. Then, the extracted essential oil was analyzed by GCMS method. By using GCMS, each chromatogram of different run times could be deduced as Citral (3.88 min), Pinene (4.63 min), Curcumene (5.34 min), Bisabolene (5.51 min), Farnesene (5.79 min), Hydrocinnamic acid, dimethyl, methyl ester (5.95 min), and 4-(1,5-dimethylhex-4-enyl) cyclohex-2-enone (6.68 min). From the chemical constituents of essential oil, it was observed that curcumene at 5.34 min was the major component. Bisabolene was the second major component in the ginger essential oil. Ginger oil helps for normal blood circulation and digestive problems. In the screening of the antioxidant activity of water and ethanolic extract from ginger, the results indicate that the ginger possesses antioxidant properties and exhibited free radical scavenging activity. It was observed that IC_{50} value of standard ascorbic acid was 2.69 µg / mL. The antioxidant activities of ethanol and water extract of ginger were 33.78 and 80.95 µg / mL. According to the comparison of IC_{50} values between standard ascorbic acid and ginger extracts, ginger possesses the rich antioxidant activity.

Keywards: Antioxidant activity, GCMS analysis, Ginger, Ginger essential oil, IC_{50}

I. Introduction

1.1 Description of Plant

Zingiber officinale Rosc., is a plant of the Zingiberaceae (ginger family) family and *Zingiber* genus, perennial herb, high 50-80 cm. Hypertrophic rhizomes, yellow-white cross-section, with a strong spicy odor. Leaves alternate, arranged in 2 rows, sessile, several clasping stems; leaf blade length 2-4 mm; leaf blade lanceolate to linear-lanceolate, 15-30 cm long, 1.5-2.2 cm wide, apex acuminate, base narrow, leaf leather sheath amphibian, glabrous. The calyx is extracted from the rhizomes and grows 15-25 cm long; the spikes are oval-shaped, 4-5 cm long; the sepals are ovate, about 2.5 cm long, pale green, with a pale yellow edge, and a small pointed tip; the calyx tube is ca. 1 cm, with 3 short tines; corolla yellow-green, tube length 2-2.5 cm, lobes 3, lanceolate, less than 2cm in length, median lobes of oblong petals oblong-ovate, shorter than corolla lobes, with purple stripes and Yellowish spots, ovate on both sides ovate, yellowish green, with purple margin; stamens 1, dark purple, anthers ca. 9 m, appendage appendages enclosing styles; ovary 3-locular, glabrous, styles 1, stigma subglobose. The majority of seeds are black. (Sutarno, *et al*., 1999)

This species mainly distributed in China's central, southeast and southwest regions. Zingiber is a creeping perennial plant native to tropical south-east Asia and cultivated in China. The aromatic, knotty rootstock is thick and fibrous, and whitish or buff in color. It produces a simple, leafy stem covered with the leaf sheaths of the lanceolate-oblong to linear

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leaves, and reaches a height of 1.25 m. The leaves are up to 30cm long and the sterile flowers are white with purple streaks and grow in small dense spikes. Ginger is a rainforest monocot about a meter high, with long, narrow leaves and spicate flowers. It has been grown in China since Antiquity. Seeds have never been found, ginger propagates through buddings from its knotty rhizome. The fresh ginger rhizome is a versatile ingredient of the far eastern cuisine, and is now commonly used in most of the world. Its flavor is lemony-balsamic and its taste is medium hot. (Thomas. 1982)

1.2 Scientific Classification

Kingdom: Plantae

Order	:	Zingiberales
Family	:	Zingiberaceae
Genus	:	*Zingiber*
Species	:	*Z. officinale*
Botanical name	:	*Zingiber officinale* Roscoe

Figure 1 Plants and rhizome of ginger

1.3 Uses of Ginger

Ginger produces a hot, fragrant kitchen spice. Young ginger rhizomes are juicy and fleshy with a mild taste. They are often pickled in vinegar or sherry as a snack or cooked as an ingredient in many dishes. They can be steeped in boiling water to make ginger herb tea, to which honey may be added. Ginger can be made into candy or ginger wine. Mature ginger rhizomes are fibrous and nearly dry. The juice from ginger roots is often used as a seasoning in Indian recipes and is a common ingredient of Chinese, Korean, Japanese, Vietnamese, and many South Asian cuisines for flavoring dishes such as seafood, meat, and vegetarian dishes. Powdered dry ginger root is typically used as a flavoring for recipes such as gingerbread, cookies, crackers and cakes, ginger ale, and ginger beer. Candied ginger, or crystallized ginger, is the root cooked in sugar until soft, and is a type of confectionery. Fresh ginger may be peeled before eating. For longer-term storage, the ginger can be placed in a plastic bag and refrigerated or frozen. (Hardon. 2001)

1.4 Health Benefits of Ginger

Evidence that ginger helps alleviate nausea and vomiting resulting from chemotherapy or pregnancy is inconsistent. It is not recommended for any clinical uses or nausea. There is no clear evidence of harm from taking ginger during pregnancy, although its safety has not been established. Allergic reactions to ginger generally result in a rash. Although generally recognized as safe, ginger can cause heartburn and other side effects, particularly if taken in powdered form. It may adversely affect individuals with gallstones and may interfere with the effects of anticoagulants, such as warfarin or aspirin. (USNCIH. 2012)

1.5 Nutritional Values of Ginger

Raw ginger is composed of 79% water, 18% carbohydrates, 2% protein, and 1% fat (table). In 100 grams (a standard amount used to compare with other foods), raw ginger supplies 80 Calories and contains moderate amounts of vitamin B6 (12% of the Daily Value, DV) and the dietary minerals, magnesium (12% DV) and manganese (11% DV), but otherwise is low in nutrient content (table). When used as a spice powder in a common serving amount of one US tablespoon (5 grams), ground dried ginger (9% water) provides negligible content of essential nutrients, except for manganese (70% DV). (USDA. 2017)

Table 1.1 Nutritional Values per 100 g (3.5 oz) of Ginger

No.	Nutrient	Composition
1	Carbohydrates	17.77 g
2	Sugars	1.7 g
3	Dietary fiber	2 g
4	Fat	0.75 g
5	Protein	1.82 g
6	Energy	333 kJ

Table 1.2 Presences of Vitamins

No.	Vitamin	Composition
1	Thiamine (B1)	0.025 mg
2	Riboflavin (B2)	0.035 mg
3	Niacin (B3)	0.75 mg
4	Pantothenic acid (B5)	0.203 mg
5	Vitamin B6	0.16 mg
6	Folate (B9)	11 μg
7	Vitamin C	22 μg
8	Vitamin E	0.26 mg

Table 1.3 Minerals

No.	Mineral	Composition
1	Calcium	16 mg
2	Iron	0.6 mg
3	Magnesium	43 mg
4	Mangnese	0.229 mg
5	Phosphorus	34 mg
6	Potassium	415 mg
7	Sodium	13 mg
8	Zinc	0.34 mg

1.6 Ginger Oil

Ginger oil has a thin consistency and is light yellow in color, with a pleasantly pungent aroma. The scent varies according to the distillation and quality of the ginger used. However, the most aromatically superior ginger oil is said to come from distilling fresh ginger root. Ginger oil benefits mostly come from its powerful mono- and sesqui-terpenoids, such as neral, geranial, 1,8-cineole, zingiberene, B-bisabolene, and B-sesquiphellandrene. It also contains a-pinene, B-pinene, camphene, linalool, borneol, y-terpineol, nerol, geraniol, and geranyl acetate. (Joseph. 1998)

The benefits of ginger for relieving pain are widely known today, and while using fresh ginger (eaten raw, grated into your vegetable juice, or steeped into tea), using ginger oil can provide these wholesome benefits as well. When used topically, ginger oil can help relieve aches and pain, as well as promote normal blood circulation. Aromatherapists also value ginger oil's soothing and warming qualities to help address digestive problems. In fact, this is one of ginger oil's most popular uses: relieving any kind of digestive upset, such as nausea, indigestion, diarrhea, gas, and even morning sickness. (Joseph. 1998)

II. Experimental

The ginger sample was collected from Hlaing Thar Yar Township, Yangon Region, Myanmar. The sample was cleaned and air-dried in room temperature. The dried samples were powdered by electric blender and stored in the air-tight container. Semi-quantitative elemental analysis of the dried powdered sample was carried out by EDXRF method. Then, the dried powdered samples were extracted with ethanol and water. The antioxidant activities of ethanol and water extract of ginger were determined by DPPH method. The extraction of essential oil from the fresh ginger was performed by steam distillation method. Then, the extracted essential oil was identified by GCMS (Trace 1300 ISQ QD) method.

III. Results and Discussion

3.1 Elemental Results of Ginger

Semi-quantitative elemental analysis of dried powdered sample of ginger was carried by EDXRF method. From the experiment, potassium and calcium were observed as considerable amounts in the sample. Potassium is very important essential element for human metabolisms. It helps to reduce the hypertension. Calcium is effective for the teeth and bone. Other elements such as magnesium, phosphorus and iron were observed as minor trace elements in ginger. These elements are essential for the metabolism of human. The experimental results are shown in Table 3.1.

Table 3.1 Elemental Composition of Ginger

No.	Analytes	Relative composition (%)
1	K	0.582
2	Ca	0.249
3	Mg	0.135
4	P	0.121
5	Fe	0.016
6	Na	0.005
7	Zn	0.003
8	Rb	0.001
9	Cu	0.001
10	CH	98.887

3.2 Extraction and Identification of Extracted Essential Oil by GCMS Method

The fresh samples of ginger were used to extract the essential oil by steam distillation method. The essential oil was extracted with n-hexane from the distillate. The extractor of essential oil of ginger is shown in Figure 2.

Figure 3.2 The essential oil extractor

Figure 3.3 GCMS Autosampler

The extracted essential oil of ginger was analyzed by GCMS Autosampler (Trace 1300, ISQ-QD, Germany) (Figure 3.3). In GCMS analysis, GC oven temperatures were assigned by four levels in the range of 80 to 280^{o}C. The increasing temperature rates were controlled by 10 to 15 ^{o}C/min; ; carrier gas, helium at a constant flow rate 1.0 mL/min. The injector temperature and mass transfer line temperature were fixed at 275 and 280 ^{o}C, respectively. The molecular masses (mass fragmentations) are arranged in 15 to 250 amu (m/z) and assigned Run time (min) in the range of 2 to 15.

From the GCMS analysis, seven peaks from the GC chromatogram were observed at 3.88, 4.63, 5.34, 5.51, 5.79, 5.95 and 6.68 min of different run times. The mass fragmentation patterns (m/z values) of each compound were matched with that of reference compounds from GCMS Libraries. By using GCMS, each chromatogram of different run times could be deduced as Citral (3.88 min), Pinene (4.63 min), Curcumene (5.34 min), Bisabolene (5.51 min), Farnesene (5.79 min), Hydrocinnamic acid, dimethyl, methyl ester (5.95 min), and 4-(1,5-dimethylhex-4-enyl) cyclohex-2-enone (6.68 min). From the chemical constituents of essential oil, it was observed that curcumene at 5.34 min was major component. Bisabolene was the second major component in the essential oil. GCMS analyzed data are shown in Figure 3.4, 3.5. (a), (b), (c), (d), (e), (f) and (g).

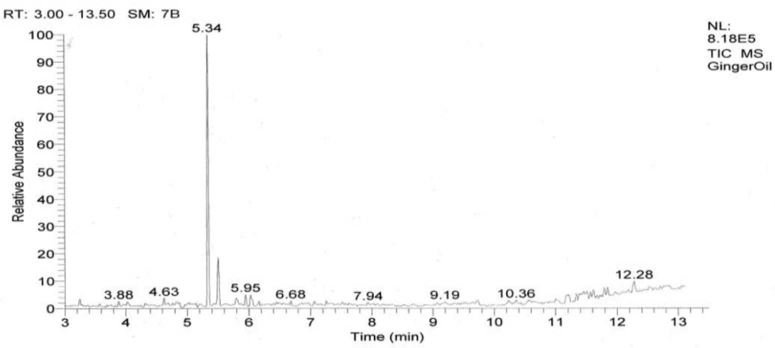

Figure 3.4 GC chromatogram of extracted essential oil of ginger

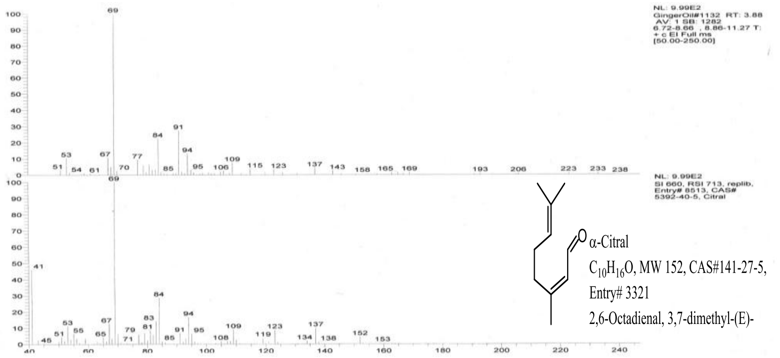

Figure 3.5 (a) Matching of fragmentation patterns of extracted essential oil and citral from GCMS data library (at 3.88 min)

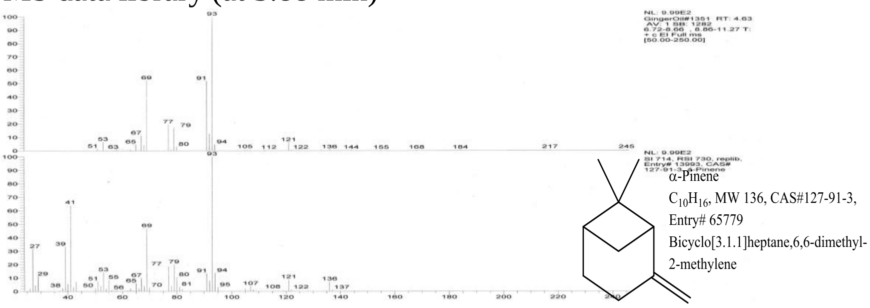

Figure 3.5 (b) Matching of fragmentation patterns of extracted essential oil and pinene from GCMS data library (at 4.63 min)

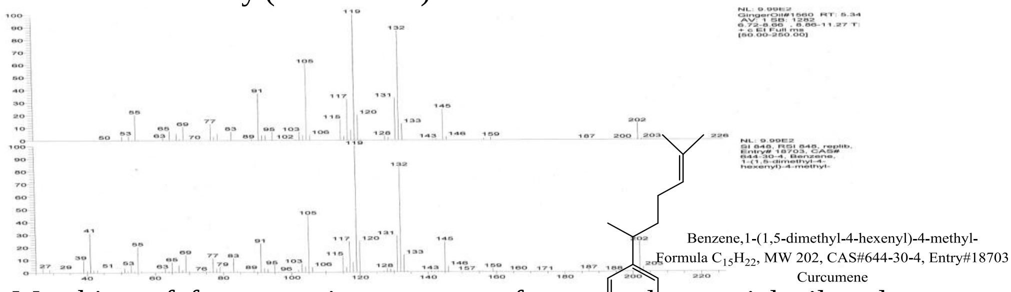

Figure 3.5 (c) Matching of fragmentation patterns of extracted essential oil and curcumene from GCMS data library (at 5.34 min)

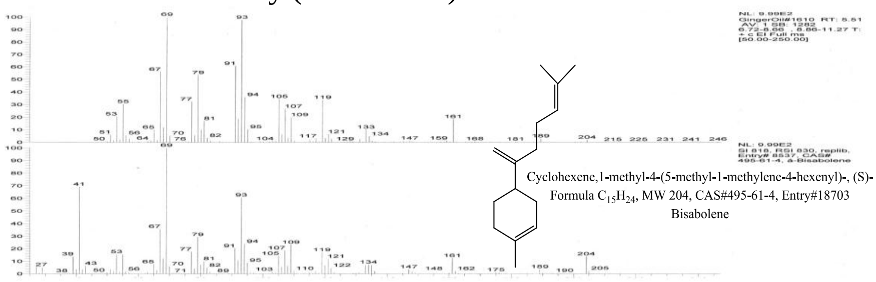

Figure 3.5 (d) Matching of fragmentation patterns of extracted essential oil and bisabolene from GCMS data library (at 5.51 min)

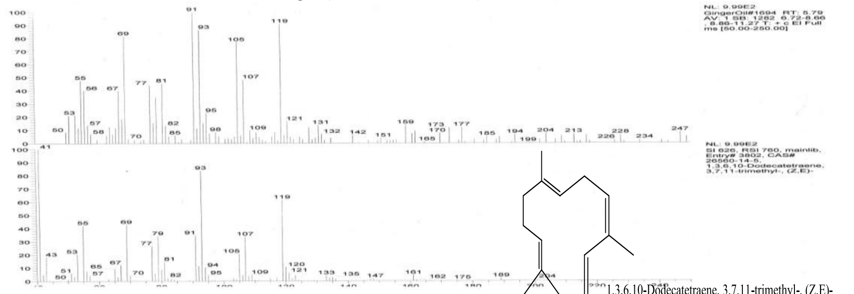

Figure 3.5 (e) Matching of fragmentation patterns of extracted essential oil and farnesene from GCMS data library (at 5.79 min)

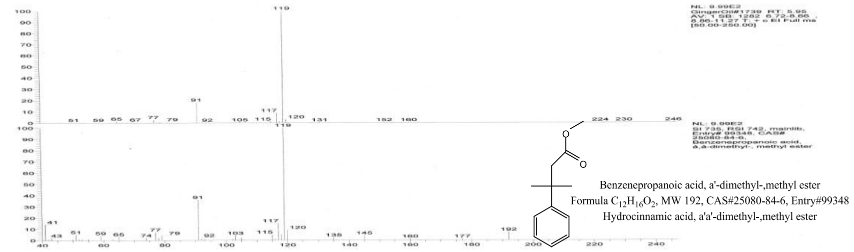

Figure 3.5 (f) Matching of fragmentation patterns of extracted essential oil and hydrocinnamic acid, dimethyl-, methyl ester from GCMS data library (at 5.95 min)

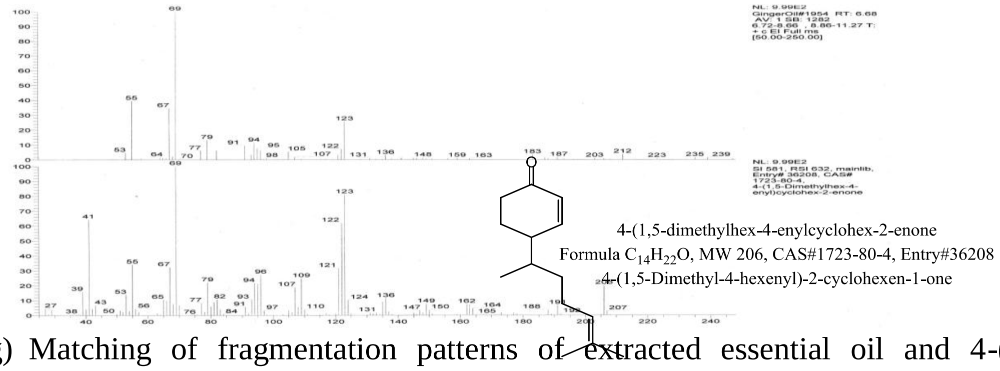

Figure 3.5 (g) Matching of fragmentation patterns of extracted essential oil and 4-(1,5-dimethylhex-4-enyl) cyclohex-2-enone (6.68 min) from GCMS data library (at 6.68 min)

3.3 Screening of Antioxidant Activity of Ginger by DPPH Method

In the screening of antioxidant activity, water and ethanol extract of ginger were determined. Ascorbic acid was used as the standard. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517nm, which is induced by antioxidants. The significant decrease in the concentration of the DPPH radical is due to the scavenging ability of the sample. The different concentrations such as 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/ mL and 6.25 µg/ mL were prepared by dilution with ethanol as solvent. The absorbance values were measured at wavelength 517nm for different concentrations of water and ethanolic extracts and the control.

From the screening of antioxidant activity, IC_{50} values of standard ascorbic acid, ethanol and water extract were observed with 2.69, 33.78 and 80.95 µg/mL, respectively. Therefore, it can be seen that ginger possesses antioxidant activity. The antioxidant activity of ethanol extract is more potent than that of water extract. The antioxidant activities of standard ascorbic acid and extract samples of ginger are shown in Table 3.2, 3.3, 3.4, Figure 3.6, 3.7, 3.8 and 3.9.

Table 3.2 Absorbance of Test Sample Solutions

No.	Concentration (µg/mL)	Ascorbic acid (Standard)	Ethanol extract	Water extract
1	6.25	0.114	0.154	0.188
2	12.5	0.091	0.124	0.187
3	25	0.061	0.122	0.167
4	50	0.010	0.053	0.130
5	100	0.001	0.023	0.083

Table 3.3 Percent Inhibition of Test Sample Solutions

No.	Concentration (µg/mL)	Ascorbic acid (Standard)	Ethanol extract	Water extract
1	6.25	40.33	24.50	7.84
2	12.5	55.09	39.21	8.33
3	25	70.09	40.19	18.13
4	50	90.09	74.01	36.27
5	100	99.5	88.72	59.31

* Absorbance of DPPH (Control) = 0.204; $\% \text{ RSA} = \frac{Abs_{DPPH} - [Abs_{Sample} - Abs_{Blank}]}{Abs_{DPPH}} \times 100$

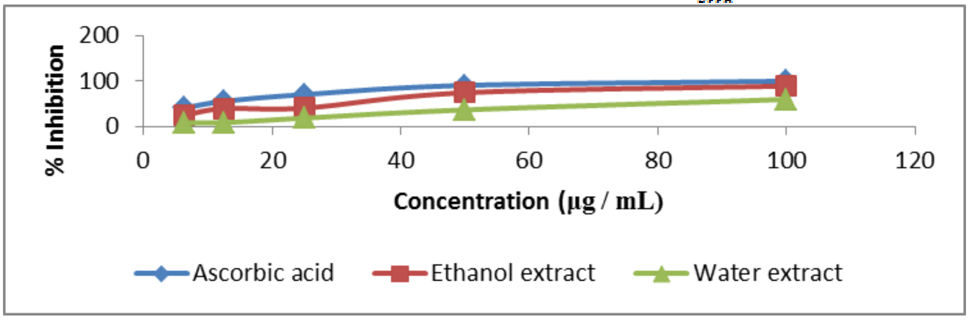

Figure 3.6 Antioxidant activities of standard ascorbic acid, ethanol and water extract with concentrations

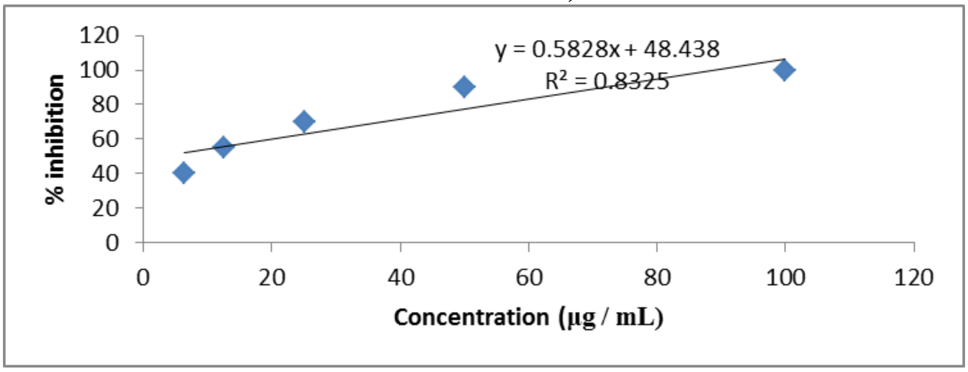

Figure 3.7 Linear regression equation for antioxidant activity (IC_{50}) of standard ascorbic acid

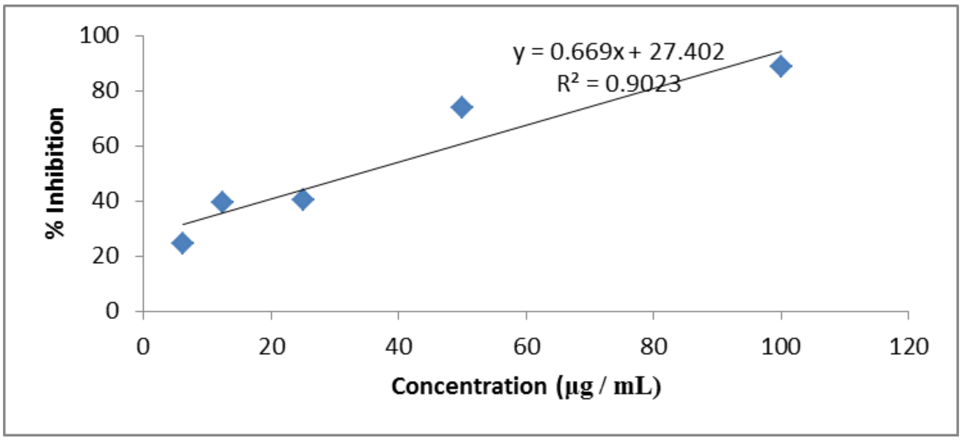

Figure 3.8 Linear regression equation for antioxidant activity (IC_{50}) of ethanol extract

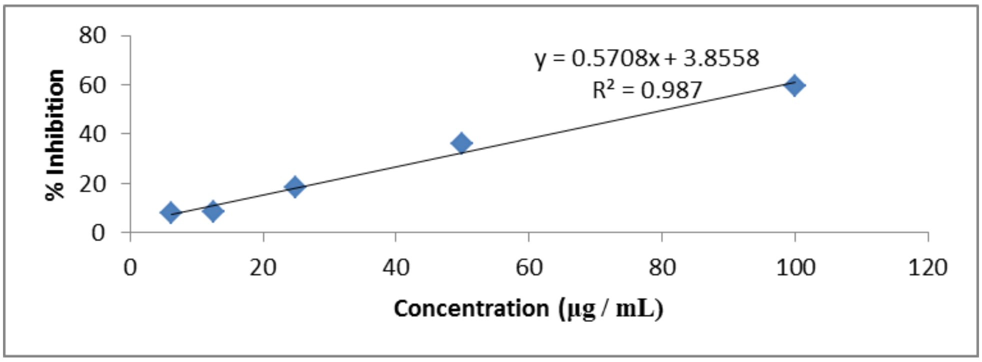

Figure 3.9 Linear regression equation for antioxidant activity (IC_{50}) of water extract

Table 3.4 The Regression Equation and IC_{50} Values of Test Samples

No.	Test Solution	Regression Equations	IC_{50} (ppm)
1	Ascorbic Acid	y = 0.582x + 48.43	2.69
2	Ethanol extract	y = 0.669x + 27.40	33.78
3	Water extract	y = 0.570x + 3.855	80.95

V. Conclusion

This research concerns with the GCMS analysis of extracted essential oil and antioxidant screening of ginger. Firstly, semi-quantitative elemental analysis of ginger was carried out by EDXRF method. These elements are essential for the metabolism of humans.

The extraction of essential oil from ginger was carried out by steam distillation method. Then, the extracted essential oil was analyzed by GCMS method. By using GCMS, each chromatogram of different run times could be deduced as Citral (3.88 min), Pinene (4.63 min), Curcumene (5.34 min), Bisabolene (5.51 min), Farnesene (5.79 min), Hydrocinnamic acid, dimethyl, methyl ester (5.95 min), and 4-(1,5-dimethylhex-4-enyl) cyclohex-2-enone (6.68 min).

In the screening of the antioxidant activity of ethanol and water extract from ginger, the results indicate that the gingers possesses antioxidant properties and exhibited free radical scavenging activity. It was observed that IC_{50} value of standard ascorbic acid was 2.69 µg / mL. The antioxidant activities of ethanol and water extract were 33.78 and 80.95 µg / mL. According to the comparison of IC_{50} values between standard ascorbic acid and ginger extracts, ginger possesses the rich antioxidant activity. Therefore, ginger may be used in the medicinal antioxidant for human health.

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