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Full Length Research Paper

Investigation of the antioxidant activity of *Illicium verum* extracts

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Illicium verum **has been widely used in many Asian countries as a spice and pharmaceutical treatment for many diseases. In this study, the antioxidant properties of the ethanol extracts fractionated by hexane, ethyl ether, chloroform and ethyl acetate, and supercritical CO_2 extracts of the plant were evaluated using 1,1-diphenyl-2-picryhydrazyl (DPPH) radical scavenging effect, the total equivalent antioxidant capacity and a reducing power assay. In addition, the amount of total phenolic content and the flavonoid content were also determined. The ethyl acetate fractions contained substantially higher levels of total phenolics (4.5 g gallic acid / 100 g dry weight) and total flavonoids (6.9 g quercetin/100 g dry weight) than the other extracts, and possessed significant antioxidant activities. The chemical components of the various extracts were analyzed by gas chromatography-mass spectrometry (GC-MS); anisyl acetone and anisyl aldehyde were evidenced to provide the slight antioxidant activities. Based on the results, it can be concluded that the ethyl acetate extracts of** ***I. verum*** **show a marked antioxidant potential and that they could be developed as a natural source of antioxidants.**

Key words: Chinese medicines, *Illicium verum*, antioxidant activity, supercritical CO_2 extraction.

INTRODUCTION

It is a well known fact that oxidation damages various biological substances and subsequently causes many diseases such as cancer, liver disease, Alzheimer's disease, premature ageing, arthritis, inflammation, diabetes, Parkinson's disease and atherosclerosis (Moon and Shibamoto, 2009). Many previous literature reports indicated that natural antioxidants possess a wide range of biological activities, including inhibition of reactive oxygen species (ROS) generation, direct or indirect scavenging of free radicals and alteration of intracellular redox reactions. As a result, antioxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and the concomitant lipid peroxidation, protein damage and DNA strand breakage. Antioxidants, which can quench reactive free radicals, can prevent the oxidation of other molecules and may thereby prevent degenerative diseases (Lee et al., 2002; Oke et al., 2009).

The number of studies on the antioxidant activity of Chinese medicinal plants has especially increased remarkably due to the potential of the plants being used as a rich and natural source of antioxidant compounds

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Abbreviations: DPPH, 1,1-Diphenyl-2-picryhydrazyl; **ROS,** reactive oxygen species; **BHT,** butylated hydroxytoluene; **SFE,** supercritical fluid extraction; **TEAC,** trolox equivalent antioxidant capacity; **ABTS,** 2,2-azinobis-3-ethylbenzthiazoline-6-sulfonic acid; **UV,** ultraviolet; **TLC,** thin-layer chromatography; **DW,** dry weight; **GAE,** gallic acid equivalent; **GC-MS,** gas chromatography-mass spectrometry.

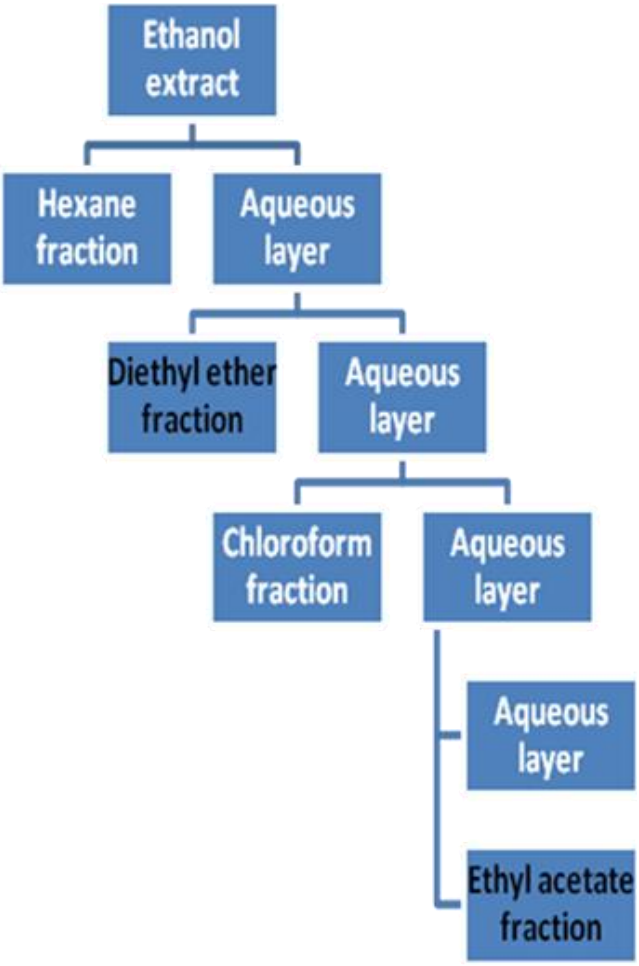


Figure 1. Fractionation of *I. verum* ethanol extracts by partition between immiscible solvents.

(Li et al., 2008; Ozen et al., 2010; Ou et al., 2003). Several studies have indicated that Chinese medicinal plants contain a wide variety of natural antioxidants such as phenolic acids, flavonoids and tannins, which possess a more potent antioxidant activity than common dietary plants (Larson, 1988; Cai et al., 2004). Compared to the synthetic antioxidants used in food processing, for example, butylated hydroxytoluene (BHT) and butylate hydroxyanisole (BHA), these substances have shown proven carcinogenic effects in living organisms (Shahidi, 2008; Nanditha et al., 2009). It is thus very important to find new sources of safe and inexpensive antioxidant substances of a natural origin.

Chinese star anise (*Illicium verum* Hook f.) is a plant originally distributed in the tropic and subtropic area of Asia and used in traditional medicine of eastern Asia. It's used in phytotherapy as well as for aromatization of pharmaceutical products, foods and cosmetics, and its usage has a long tradition. The fruits are commonly used as a spice and pharmaceutical treatment for flatulence, spasmodic pain and colics. The oil of star anise is employed topically to treat rheumatism and otalgia, and is also used as an antiseptic (De et al., 2002).

In previous study, it has documented that *I. verum* Hook presents antimicrobial, antifungal, antiinflammatory, antiallergic and anticancer effects (Yang et al., 2010). The major components of the essential oil of *I. verum* Hook are (*E*)-anethole, limonene, linalool and α-pinene (Wang et al., 2006). However, the antioxidant activity and composition information is not much available on the antioxidant nature of this plant. The aim of the present study was to investigate the antioxidant activity of the traditional solvent and supercritical fluid extracts of the plant, as well as to determine the active ingredients of the extracts.

MATERIALS AND METHODS

Plant extract preparation

The fruits of star anise (*I. verum*) were purchased from local herb suppliers which were authenticated by the Department of

Traditional Chinese Medicine, Kaohsiung Medical University Chung-Ho Memorial Hospital, Taiwan. The raw materials were washed, air-dried, grounded and preserved in a refrigerator before extraction. For the ethanol extraction, the dried plant fruits (100 g) was extracted with 500 ml of 95% ethanol overnight by shaking it in an incubator set at 200 rpm and 37°C as described in a previous study (Liang et al., 2008). The crude extracts were successively partitioned with hexane, ethyl ether, chloroform and ethyl acetate based on polarity. The process followed the sequence depicted in Figure 1. For the supercritical fluid extraction (SFE), the ground powders (5 g) with a grain size of 1 to 2 mm were first evently immersed in the extractor for 5 min and then dynamically extracted until the weight ratio of the CO_2 flow to the loaded samples was roughly equal to 30. Solvent flow rates were approximately 3.1 to 4.0 ml/min. The high pressure set-up from ISCO, model 260D, was employed to conduct the extraction. The CO_2 effluent from the extractor was depressurized through a heated capillary and trapped in ethanol. Each extract was dried by evaporation, and then subjected to the antioxidant assay.

Antioxidant activity

As emphasized in many previous studies (Wod et al., 2006), no single method is adequate for determining the antioxidant capacity of food as different methods obtain widely diverse results. Therefore, several methods based on different mechanisms must be used to determine the antioxidant activities. In this study, we applied assays of 1,1-diphenyl-2-picryhydrazyl (DPPH) radical scavenging activity, 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical scavenging ability, and the reducing power to each extract.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical assay was determined as a previous study (Liang et al., 2008), with a few minor modifications. Briefly, 1 ml of different concentrations of the test samples (alcohol crude extracts, various fractionated extracts, or SFE extracts) was mixed with 1 ml of 0.5 mM DPPH in ethanol. Mixtures were vigorously shaken and left for 30 min in the dark. Absorbance was measured at 517 nm using ethanol as a standard. 1 ml of 0.5 mM DPPH diluted in 2 ml of ethanol was used as a control sample. Inhibition of the DPPH radicals was calculated using the following equation:

$$I\% = \left[1 - \frac{X_1 - X_2}{X_3}\right] \times 100\%$$

where X_1 is the absorbance of the tested sample (in a DPPH solution), X_2 is the absorbance of the tested samples without DPPH and X_3 is the absorbance of the control sample (which contains all reagents except for the test samples). The IC_{50} value represents the concentration of the tested sample that caused 50% inhibition.

Trolox equivalent antioxidant capacity (TEAC) assay

The assay was measured by the method described by Miller et al. (1993) with a few modifications. Peroxidase (4.4 units/ml, Sigama, MO, USA), H_2O_2 (50 µM , Merck, Germany), ABTS (100 µM, Sigama, MO, USA) and 1 ml phosphate buffer (0.1 M , pH 6.0) were mixed and kept in the dark to react for 1 h for reaction. 1 ml of the plant extract was subsequently added and reacted for 10 min at room temperature, and then the absorbance was measured at 734 nm. A trolox solution (final concentration 0-15 µmole/L) was used as a reference standard with a correlation coefficient of $R^2 = 0.9995$. The results were expressed as mmole Trolox/100 g dry weight (DW) of the plant material.

Reducing power test

The reducing power was evaluated according to the method described by Duh et al. (2004). 1 ml of the various extracts (g/ml), phosphate buffer (0.2 M, pH 6.6, 0.5 ml), and $K_3Fe(CN)_6$ (1% *v/w*, 2.5 ml) were mixed and incubated at 50°C for 20 min. The test tube was cooled on ice and 0.5 ml at 10% of trichloroacetic acid (TCA) was added. After centrifugation at 3000 × g for 10 min, 1 ml of aliquot supernatant mixed with 1 ml distilled water and 0.1 ml ferric chloride (0.1%) was added and reacted for 14 min. Finally, the absorbance at 700 nm was measured; increased absorbance of the reaction mixture indicated increased reducing power.

Determination of total phenolics concentration

The total phenolic content of the plant extracts was determined by the Folin-Ciocalteau method (Slinkard et al., 1997). Briefly, 200 µl of diluted samples were added to 1 ml of a 1:10 diluted Folin-Ciocalteau reagent. After 4 min, 800 µl of a saturated sodium carbonate solution (75 g/L) was added. After 2 h of incubation at room temperature, the absorbance at 760 nm was evaluated using a spectrophotometer. Gallic acid (0-500 mg/L) was used for calibration of the standard curve and the correlation coefficient was $R^2 = 0.9965$. The results were expressed as gram gallic acid equivalent (GAE)/100 g DW of the plant material.

Determination of total flavonoids concentration

Many reports have determined that the antioxidant activity of plant extracts is mainly ascribable to the concentration of phenolic compounds. The Folin-Ciocalteu method measures the reduction of the reagent by phenolic compounds via the formation of a blue complex that can be measured at 760 nm against gallic acid as a standard. The total flavonoid content was established in the reaction with aluminum chloride using a colorimetric method (Chang et al., 2002). Briefly, 1 ml of each extract was shaken for 1 min and 0.1 ml of 10% aluminum nitrate, 0.1 ml 1M potassium acetate and 3.8 ml of methanol was added. After 40 min at room temperature, the absorbance was measured on the ultraviolet (UV)/Visible spectrophotometer at 415 nm. The flavonoids were assessed by plotting the quercetin calibration curve (10-200 mg/L). The equation for the quercetin calibration curve was $Y = 0.0101 \cdot X - 0.0632$ (where X = concentration of quercetin equivalents expressed as milligrams of quercetin per 100 g of dry weight of the plant material; Y = measured absorbance). The correlation coefficient was $R^2 = 0.9967$.

Determination of the chemical compositions

Thin-layer chromatography (TLC) analysis

1 g of the tested sample was dissolved in 1 ml of dimethyl sulfoxide (DMSO). 2 µl of each sample solution were applied onto a TLC plate (20 × 20 cm, Merck, silica gel 60 F_{254}) in bands of 2 cm. The TLC plate was developed with an n-hexane/ ethyl acetate mixture (2/8; v/v) as a mobile phase. The spots were visualized by UV irradiation at 358 nm or 418 nm.

GC-MS analysis

The chemical components were identified by gas chromatography-;

Table 1. Antioxidant activities of the extracts of *I. verum*.

Extract	DPPH IC_{50} [1]	TEAC [2]	Reducing Power [3]	TPC [4]	Flavonoid [5]
Ethanol crude extract	80.64 ± 13.08	15.82 ± 0.061	0.7 ± 0.01	3.19 ± 0.21	6.63 ± 0.10
Hexane fraction	3457.50 ±150.18	0.12 ± 0.013	-	0.45 ± 0.07	-
Ethyl ether fraction	57.43 ± 7.03	26.75 ± 0.022	1.9 ± 0.02	4.19 ± 0.53	4.84 ± 0.11
Chloroform fraction	310.76 ±12.33	25.73 ± 0.104	0.2 ± 0.00	3.29 ± 0.02	0.91± 0.01
Ethyl acetate fraction	38.60 ± 0.55	26.95 ± 0.023	1.2 ± 0.02	4.49 ± 0.12	28.63 ± 0.87
Aqueous fraction	389.45 ± 3.14	18.24 ± 0.275	0.4 ± 0.01	4.41 ± 0.22	3.41± 0.05
SFE extract	-	-	-	-	-
Trans-anethole	-	-	-	-	-
Anisyl adehyde	446.27 ±13.78	0.32 ± 0.61	0.4 ± 0.00	-	-
Anisyl acetone	1253.75 ± 3.14	0.26 ± 0.10	0.07 ± 0.00	-	-
Anisyl alcohol	223.26 ±17.33	0.18 ± 0.24	0.30 ± 0.01	-	-
BHT	27.09 ± 2.93	1.66 ± 0.11	0.34 ± 0.01		

-, no activity; **[1],** DPPH radical scavenging effect, ppm; **[2],** trolox equivalent antioxidant capacity , mmol Tolox/100 g dry weight; **[3],** reducing power, absorbance 700 nm; **[4],** total phenolic content, g GAE/100 g dry weight; **[5],** total flavonoid content, mg quercetin/100 g dry weight.

mass spectrometry (GC-MS) (GC, Agilent 6890N; MS, Agilent 5970 DB-5 column, 60 m × 250 µm × 0.25 µm). The GC analysis was set at injector temperature (280°C), temperature program: 80°C (hold 2 min), 80-140°C (3°C min^{-1}), 140°C (hold 5 min) and 140-270°C (4°C min^{-1}). The percentage composition of the tested sample was computed by the normalization method from the GC peak areas, without using correction factors.

Statistical data analysis

The data obtained from the antioxidant assays were presented as means of three replicate determinations ±standard deviation (SD). The data were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between means was determined with SPSS software (v.12, SPSS, USA) at a significance level of 0.05.

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents

The amount of total phenolics varies in the different extracts ranging from 0 to 4.49 g GAE per 100 g DW in the plant materials, as shown in Table 1. The phenolic content of the ethanol crude extract was 3.19 g GAE/100 g DW. Following the partition distribution, the extracted fractions obtained from ethyl acetate (4.49 g GAE/100 g DW), water (4.41 g GAE/100 g DW) and ethyl ether (4.19 g GAE/100 g DW) were found to possess the highest total phenolic content among the six extracts, whereas the hexane fractions and the SFE extracts contained the least phenolic compounds. These results indicate that a solvent of higher polarity provides better solubility for phenolic substances from plant materials (Mohdaly et al., 2010). Flavonoids possess a broad spectrum of chemical and biological activities, including antioxidant and antimicrobial properties. As shown in Table 1, the highest flavonoid content was observed in the fractions of ethyl acetate (28.63 g quercetin/100 g DW), followed by the ethanol crude extracts (6.63 g quercetin/100 g DW) and the ethyl ether fractions (4.84 g quercetin/100 g DW). However, extracts obtained with solvents of lower polarity, such as hexane and supercritical CO_2 fluid had the lowest flavonoid content.

1,1-Diphenyl-2-picrylhidrazyl (DPPH) radical assay

The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity. Free radical scavenging properties of the SFE and organic solvent extracts are presented in the Table 1. Lower IC_{50} value indicates higher antioxidant activity. The extracts obtained from the ethyl ether fractions and the ethyl acetate fractions exhibited a remarkable antioxidant activity with IC_{50} values of 57.43 and 38.60 ppm, respectively. However, the hexane fractions and SFE extracts display a poor DPPH scavenging ability.

Trolox equivalent antioxidant capacity (TEAC) assay

The decolorization of $ABTS^+$ radicals reflects the capacity of an antioxidant species to donate electrons or hydrogen atoms to inactivate the radical species. The $ABTS^+$ radical scavenging potential of the various extracts is presented in Table 1. The neutralizing ability of $ABTS^+$ radicals for the extracts of ethanol, the hexane, ethyl ether, chloroform, ethyl acetate and the aqueous fractions were 15.82, 0.12, 26.75, 25.73, 26.95 and 18.24 mmol

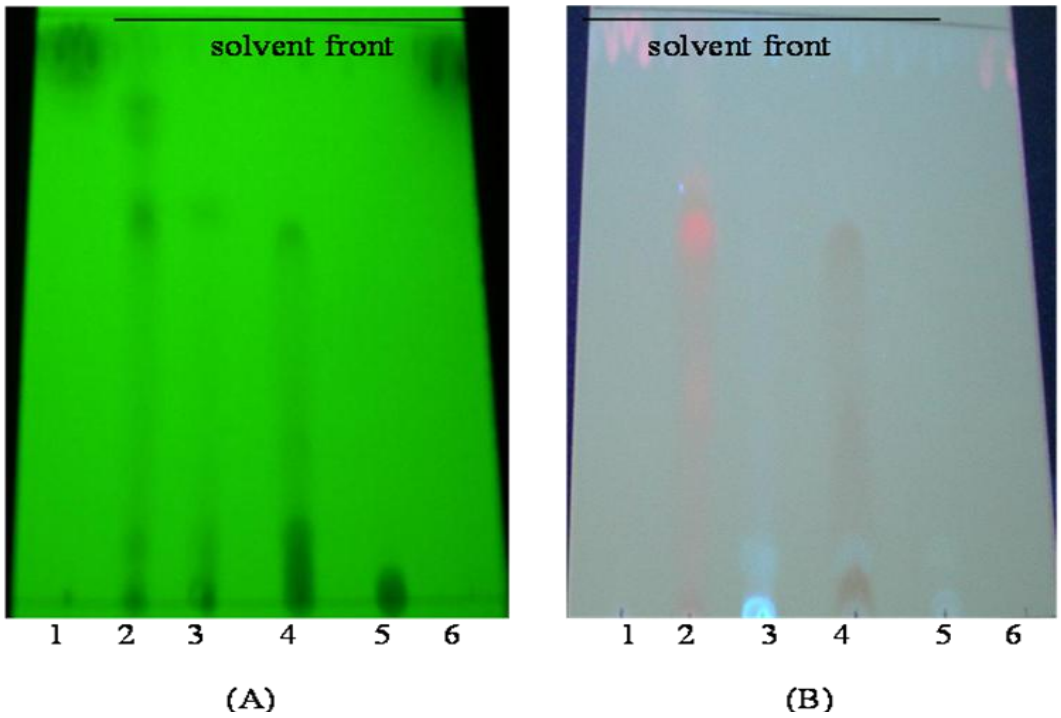


Figure 2. Thin-layer chromatograms of the extracts developed in n-hexane/ethyl acetate (2:8; v/v) solvent: (A), TLC chromatogram visualized under ultraviolet light (358 nm); (B), TLC chromatogram visualized under ultraviolet light (418 nm). 1, Hexane fraction; 2, diethyl ether fraction; 3, chloroform fraction; 4, ethyl acetate fraction; 5, aqueous fraction; 6, SFE extract.

Trolox/100 g DW, respectively. The SFE extract did not show any $ABTS^{+}$ radical scavenging ability. The results indicate that the ethyl ether and ethyl acetate fractions are capable of scavenging free radicals via an electron- or hydrogen donating mechanisms and thus, should be able to prevent the initiation of deleterious free radical mediated chain reactions in susceptible matrices such as biological membranes. Based on the results for the total phenolic and flavonoid content, phenolics and flavonoids of the extracts probably were involved in the free radical scavenging mechanism. The obtained results show the capability of the extracts to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage.

Reducing power test

The ethyl ether fraction (A_{700} = 1.9) and ethyl acetate fraction (A_{700} = 1.2) exhibited higher activities than the other extracts as shown in Table 1. The antioxidant activity was even higher than for the standard BHT (A_{700} = 0.34). Similar to results obtained in the radical scavenging analysis, the total phenolics and flavonoids from *I. verum* showed a positive correlation with their reducing capacity.

Thin-layer chromatography (TLC) analysis

Each extract fraction and the SFE extract were subjected to a silica gel TLC analysis to characterize their chemical profile. The presence of polyphenolic and flavonoid compounds in the extracts was evidenced by the intense fluorescence produced under UV_{358} and UV_{418} light (Figure 2). The results of the TLC showed that the fractions of ethyl ether and ethyl acetate contained higher concentration of flavonoid compounds (R_f 0.63 - 0.67) compared to other extracts. The hexane fraction and SFE extracts contained a higher amount of trans-anethole (R_f 0.92).

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of the extracts led to the identification at least eight major constituents, which are listed in Table 2 along with their quantitative data. The major component in the n-hexane fraction (Figure 3A), the ethyl ether fraction (Figure 3B) and the SFE extracts was mainly trans-anethole (Figure 3F), with an area percentage of 59, 47 and 100%, respectively. 5-methoxy-2-methyl-benzenamine (43%), phenol (37%), and anisyl acetone (42%) were the most abundant components in the

Table 2. Composition of the extracts from *I. verum*.

Extract	Major chemical component	Peak area (%)[#]
n-hexane fraction	1. Trans-anethole	59.08
	2. Anisyl aldehyde	12.91
	3. Anisyl acetone	5.23
	4. 4-(2-propenyl)-phenol	22.78
Ethyl ether fraction	1. Trans-anethole	47.04
	2. Anisyl aldehyde	15.02
	3. Anisyl acetone	19.04
	4. Anisyl alcohol	11.70
Chloroform fraction	1. Anisyl acetone	9.22
	2. 5-Methoxy-2-methyl-benzenamine	43.24
Ethyl acetate fraction	1. Anisyl acetone	19.51
	2. Phenol	36.59
	3. Benzenecarboxylic acid	8.37
Aqueous fraction	1. Anisyl acetone	42.49
	2. Phenol	8.62
	3. Benzenecarboxylic acid	16.14
SFE extract	1. Trans-anethole	100.00

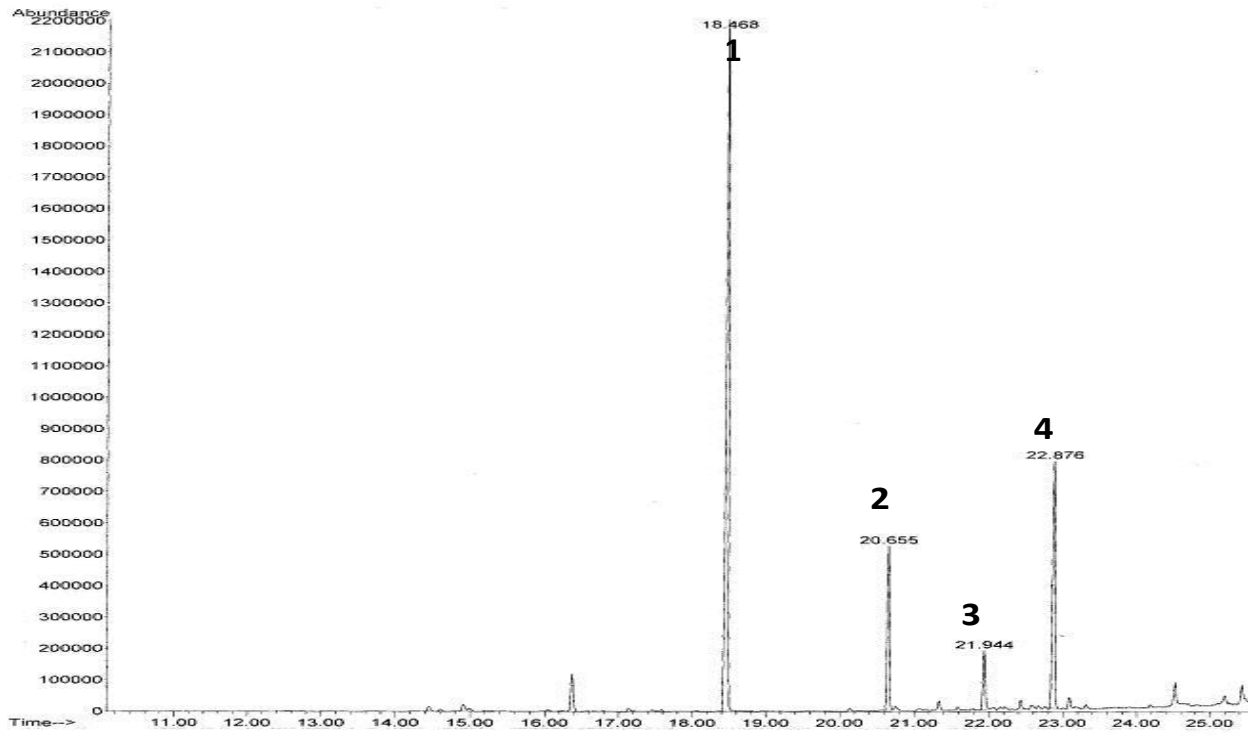
[#] The area % are determined by Enhanced Data Analysis and the compounds are determined by database (wiley7N).

chloroform (Figure 3C), ethyl acetate (Figure 3D) and aqueous fractions (Figure 3E), respectively.

The antioxidant capacities of the plant extracts highly depend on the composition of the extracts and conditions of the test system. In literature reports the antioxidant values correlate well with both the phenolic and the total flavonoid contents (Tsai et al., 2008). In this study, we found a correlative relationship between both the total phenolic and the flavonoid compounds and all three antioxidant assays in the ethyl acetate and ethyl ether fractions of *I. verum*. The results obtained verified that the phenolics and flavonoids in *I. verum* made the major contribution to the antioxidant ability.

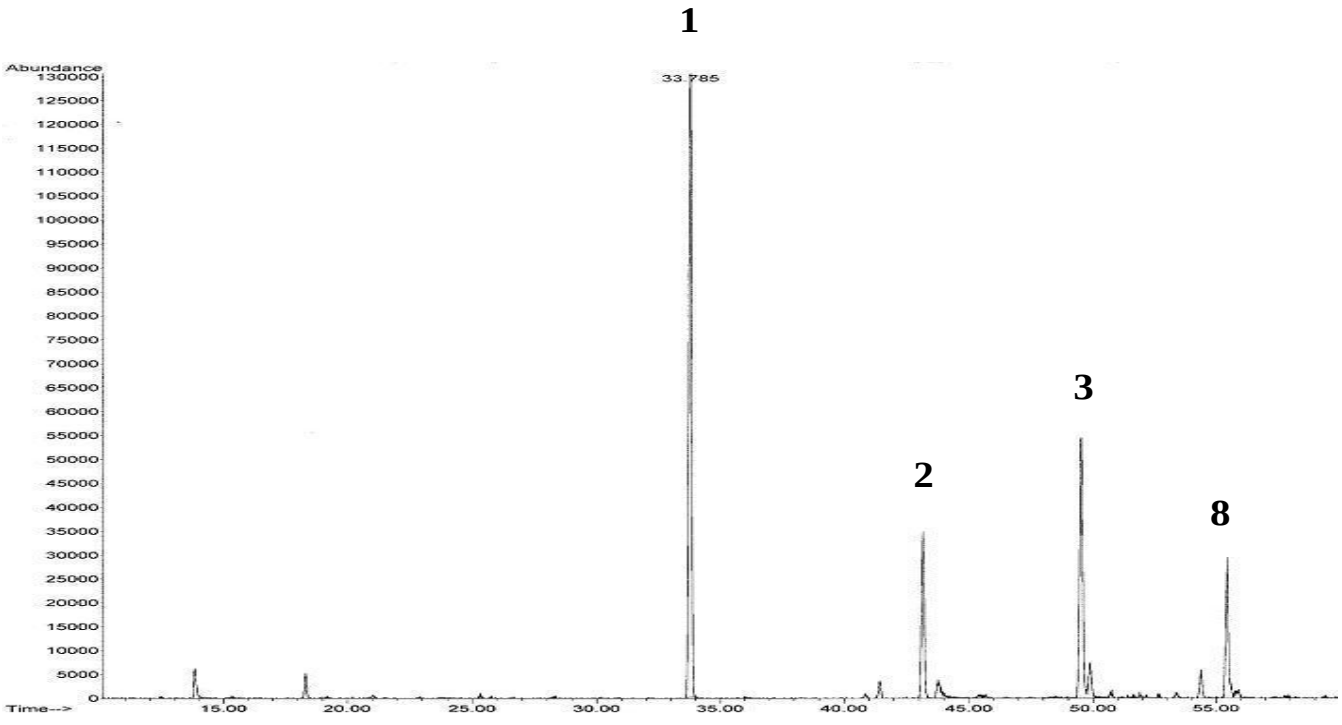
Plant extracts with proven antioxidant activity are usually composed of a phenolic moiety, for example cumarins and tocopherols. Organic acids, carotenoids and tannins can also be present and act as antioxidants or have a synergistic effect with phenolic compounds (Dapkevicius et al., 1998). The antioxidant activity of phenolics is mainly due to their redox property, which allows them to act as reducing agents, hydrogen donators and singlet oxygen quenchers (Ozen et al., 2011). Natural polyphenols have chain-breaking antioxidant activities and are believed to prevent many degenerative diseases, including cancer and atherosclerosis (Roginsky, 2003). In the present work, the ethyl ether and ethyl acetate fractions showed promising results in terms of total phenolic content and antioxidant activities on the DPPH and TEAC assays. However, the aqueous fraction and the chloroform fraction showed only a weak correlation between the phenolic content and antioxidant potency. This suggests that in addition to phenolic compounds, some other substances, such as flavonoids, may also be responsible for the observed antioxidant activity (Kaneria et al., 2009). The results of this study clearly indicate that phenolics and flavonoids are important components of *I. verum*, and its free radical scavenging and antioxidant effects could be attributed to presence of these valuable constituents.

Amongst all the various extracts analyzed, eight major components were identified by GC-MS analysis. Trans-anethole, anisyl aldehyde, anisyl alcohol and anisyl acetone were present in a higher concentration than other phytoconstituents. As reported previously in the literature, (*E*)-anethole was most abundant component in *I. verum* and evidenced to possess antimicrobial properties (Yang et al., 2010). A comparison of the antioxidant activities of the extracts and the major components showed that the ethyl acetate fraction exhibited the highest antioxidant capacity among all the extracts. The test standards revealed poor antioxidant abilities, except for anisyl aldehyde and anisyl alcohol, which possess a slight antioxidant effect. Obviously, the antioxidant substances should be phenolic and flavonoid



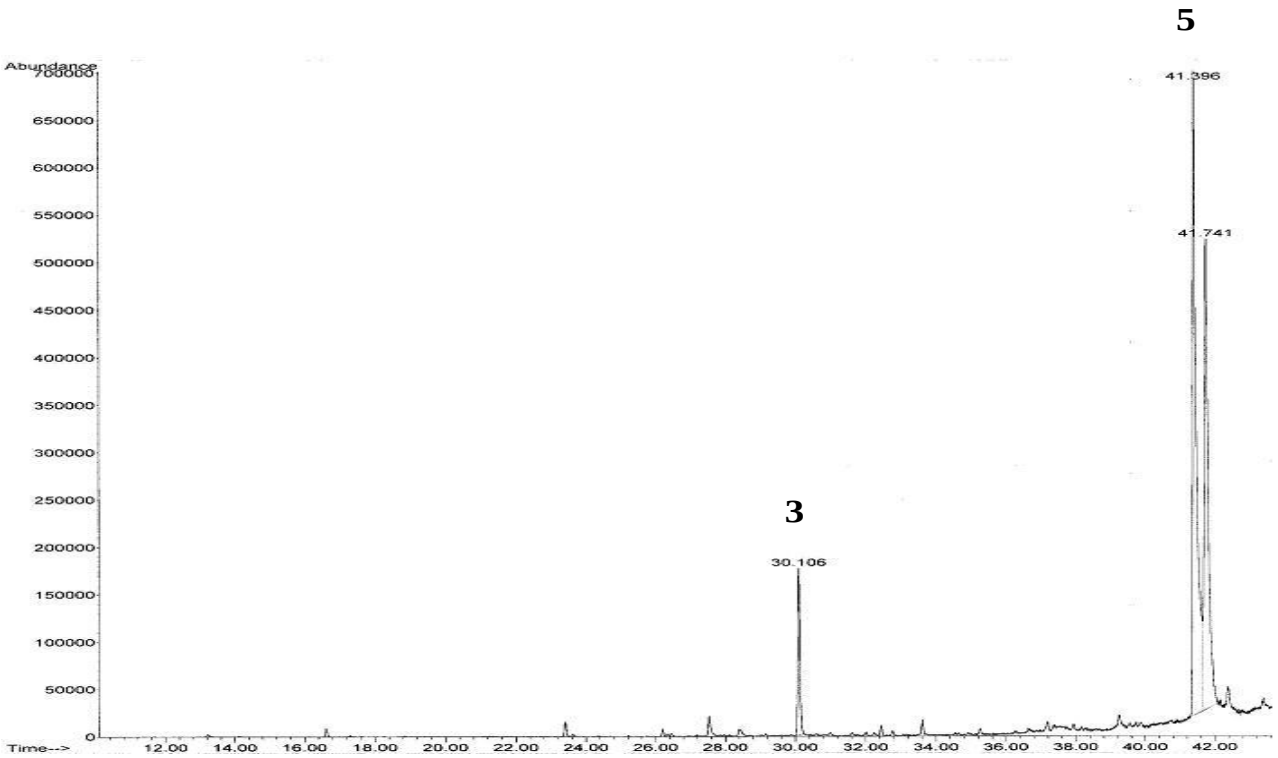
(A)

Figure 3. Gas chromatography-mass spectrometry profile of the various extracts of *I. verum*. **(A),** n-hexane fraction;



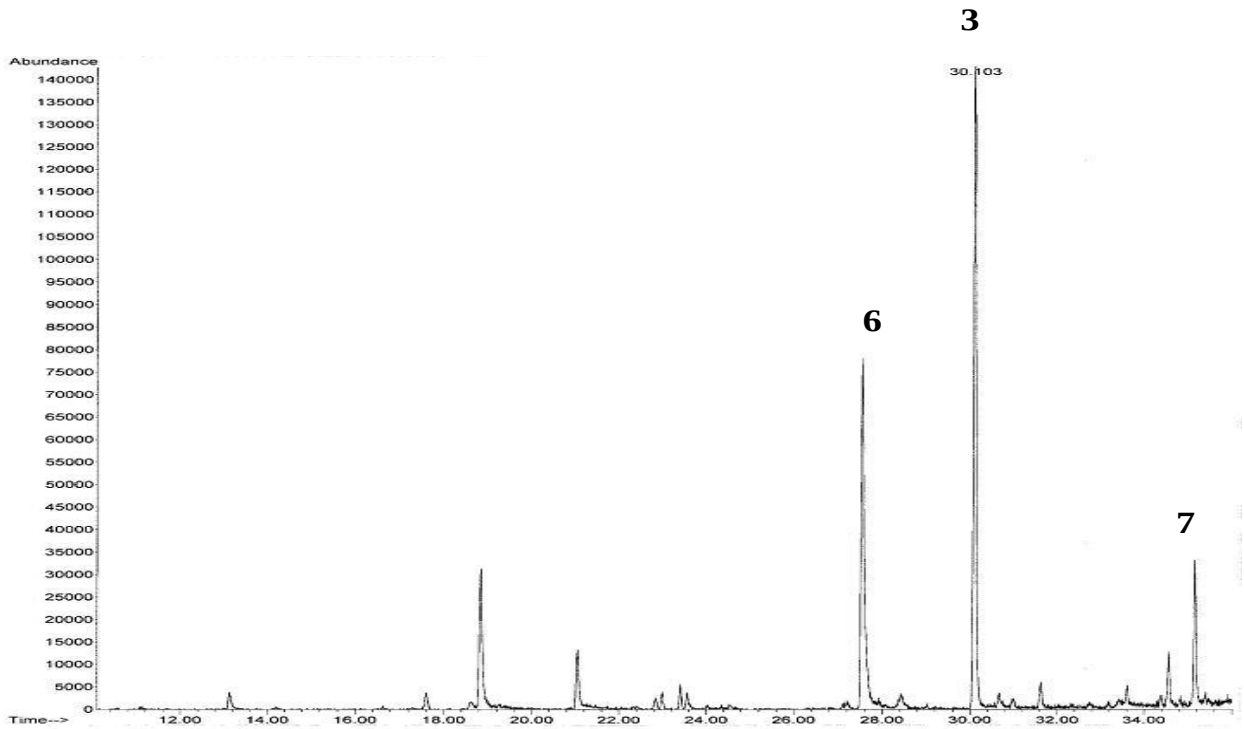
(B)

Figure 3. (B), ethyl ether fraction;



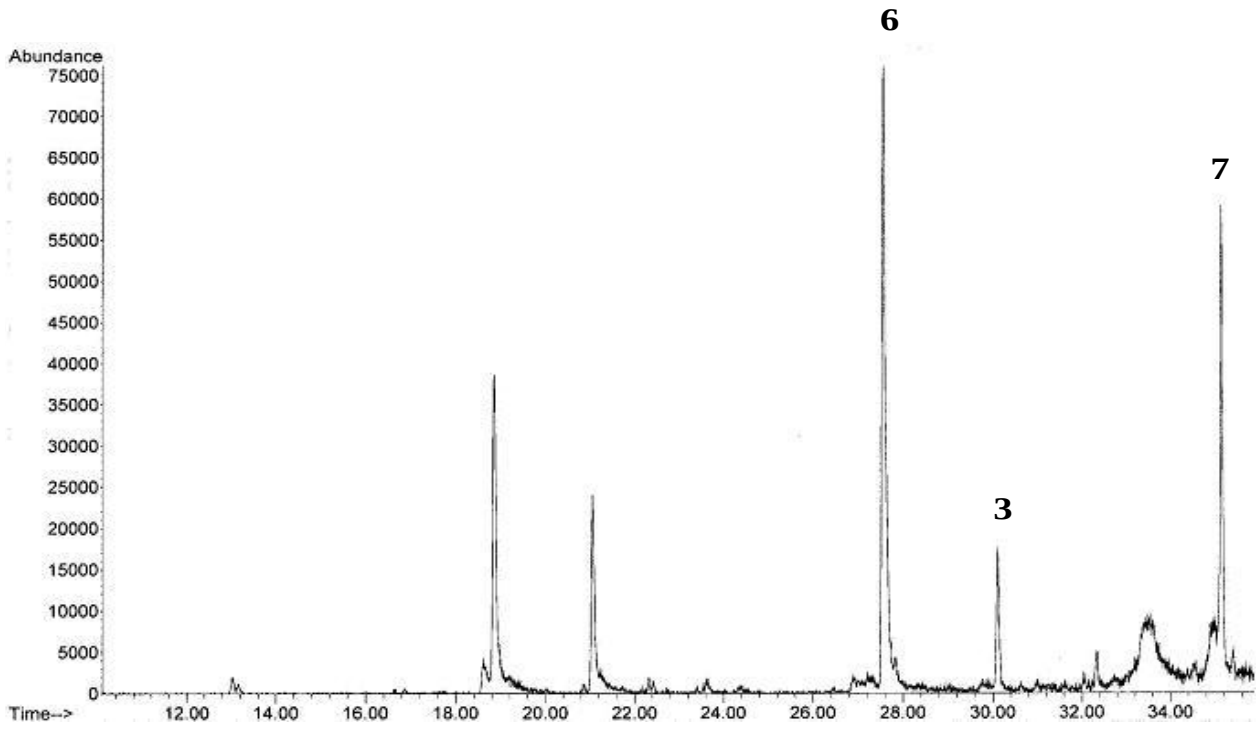
(C)

Figure 3. (C), chloroform fraction;



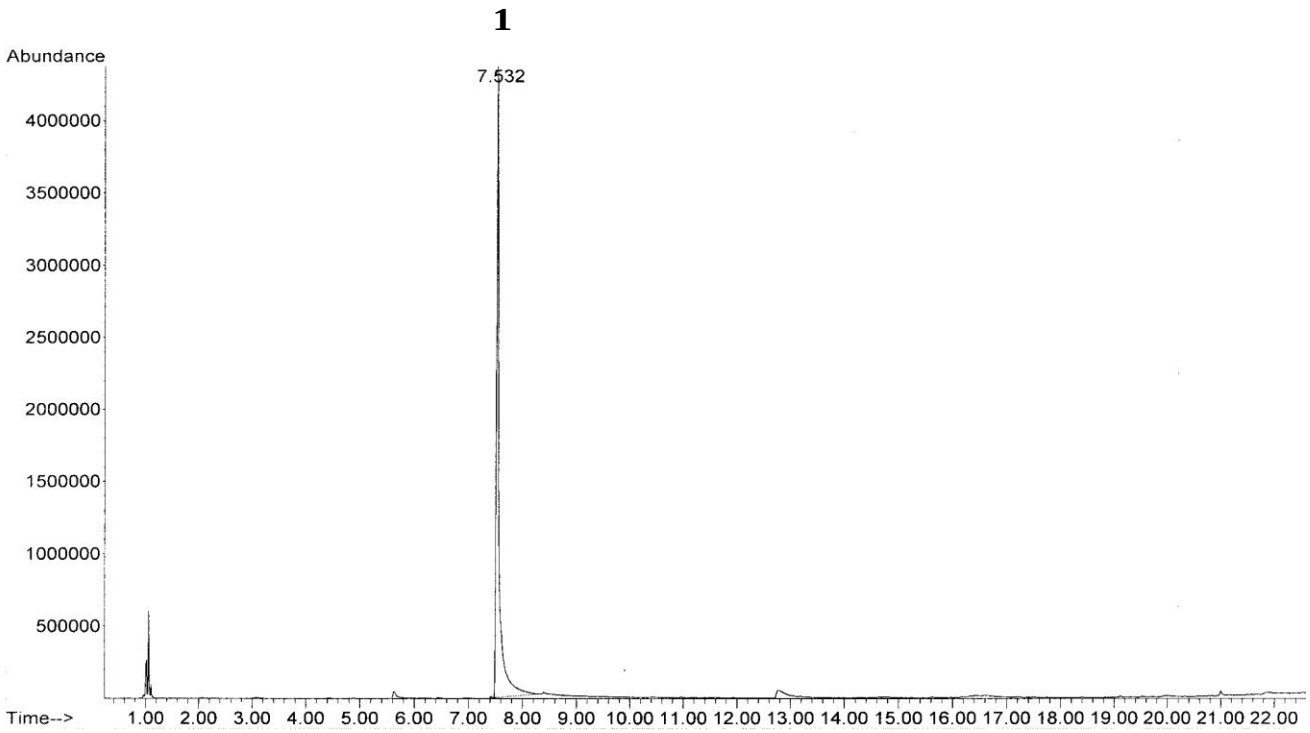
(D)

Figure 3. (D), ethyl acetate fraction;



(E)

Figure 3. (E), aqueous fraction;



(F)

Figure 3. (F), SFE extract; 1, trans-anethole; 2, anisyl aldehyde; 3, anisyl acetone; 4, 4-(2-propenyl)-phenol; 5, 5-methoxy-2-methyl-benzenamine; 6, Phenol; 7, Benzenecarboxylic acid; 8, anisyl alcohol.

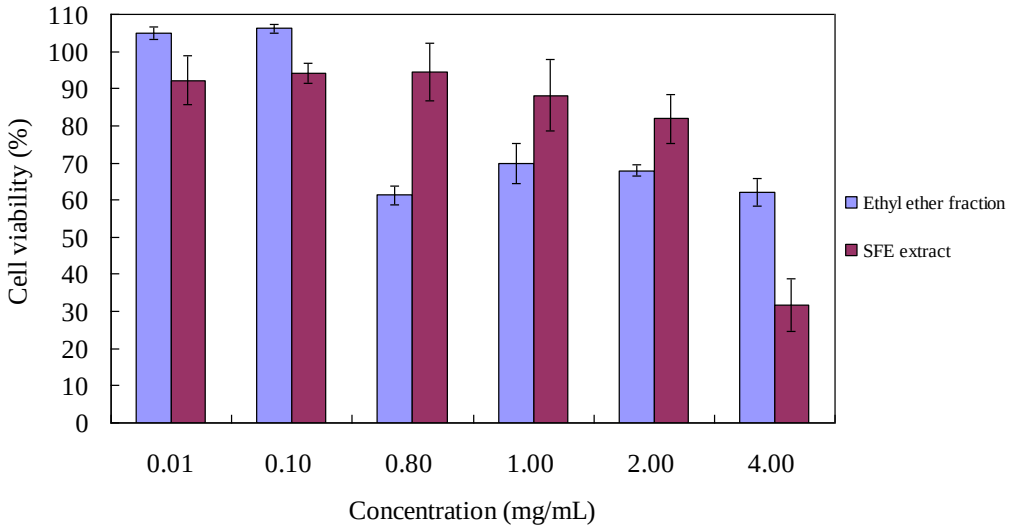


Figure 4. Cell viabilities of human dermal fibroblasts treated with ether fraction and SFE extracts obtained from *I. verum*.

compounds with a mild higher polarity but not those components identified in the extracts. This finding is similar to the findings in Padmashree's report that total phenol and total flavonoids correlated significantly with β-carotene bleaching in star-anise (Padmashree *et al.*, 2007). Especially (*E*)-anethole, the major component found in the hexane fractions and SFE extracts showed a poor antioxidant activity. The Padmashree and coworkers study also determined that the ethanol/water extracts of star-anise have a higher antioxidant activity than the petroleum ether extracts.

In our previous study (Yang et al., 2010), we used human dermal fibroblasts as an *in-vitro* model to measure cytotoxic effects with the MTT assay. The active extracts of the ethyl ether fractions and non-active extracts of SFE obtained from *I. verum* showed relativity low cytotoxicity (Figure 4), with a viability above 50% at concentrations of 4 mg/ml and 2 mg/ml, respectively. The results mean that the active extracts obtained from *I. verum* exhibit a low toxicity for mammals, a fact supported by the literature (Budavari et al., 1989). The ethyl acetate extract from *I. verum* should therefore, be essentially safe for use in humans. Based on the results of this study, it can be concluded that the *I. verum* extracts have a promising potential as a source of natural antioxidants. The identification and isolation of the active compounds from ethyl acetate fractions is suggested for further investigation. They could be used as preservative ingredients in the food or pharmaceutical industry, provided that any resulting organoleptic effects are accepted.

Conclusion

In the present study, we have demonstrated the antioxidant activities of various extracts from *I. verum*. The results underline that the ethyl acetate fractions possess a free radical scavenging ability and reducing power effect superior to those of other extracts. The strong correlation between its DPPH and TEAC values with those obtained from the reducing power assay implied that the antioxidants in the extracts were capable of scavenging free radicals and reducing oxidants. The antioxidant components of *I. verum* were also characterized by thin-layer chromatography and GC-MS in this study. A significant relationship between the antioxidant capacity and the total phenolic and flavonoid content was found. These results suggest that phenolic and flavovoid compounds contribute to the antioxidant activities of the ethyl acetate fractions. This study reveals that *I. verum* may potentially be an effective source of natural antioxidants.

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