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Extraction and Antioxidative Activity of Essential Oil From Star Anise (*Illicium verum*)

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ABSTRACT

Star anise (*Illiciumverum*) essential oil was extracted using solvent extraction method. The extraction yields and antioxidant activities of essential oils at different extraction times (1, 3, 5, 7 and 9 days) and temperatures (30, 40, 50, 60, 70 °C) were studied. The results showed that the highest yield of essential oil was 8.56 % by extracting star anise at 60 p C for 7 days.The antioxidant activities of the extracted star anise essential oils were investigated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay on Thin Layer Chromatography (TLC) plates and DPPH radical scavenging method. The results showed that at least two different bands with antioxidant activity with different polarity were appeared on the TLC plates after spraying with DPPH and incubated for 30 minutes. The highest antioxidant activity of star anise essential oil was obtained when the sample was extracted at 60 p C for 1 day (EC_{50} value = 0.089±0.05 mg/ml). HPLC analysis showed that the concentration (%) of trans-Anethole present in the essential oils extracted at varied extraction times and temperatures was ranged from 77.29 % to 91.87 %.Gas Chromatography-Mass Spectrometry (GC-MS) analysis was also done on a sample of star anise essential oil and a distinctive peak at retention time 13.84 minutes with peak area 100% was found to be Estragole compound. Anethole compound was also found to be present at two peaks.

Key words: solvent extraction, star anise essential oil, Extraction yield, antioxidant activity, trans-Anethole.

INTRODUCTION

Long term and extensive use of synthetic antioxidant such as butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA) have been proven to cause carcinogenic effects in living organisms[1-3]. Due to the potential side effects of synthetic antioxidants, essential oil which derived from organic products can be served as an alternative source for the further improvement of synthetic antioxidant. Star anise (*Illiciumverum*) is a small star-shaped fruit of an evergreen of the Illiciaceae family[4]. This fruit is classified as a spice and it looks like a symmetrical eight-pointed star[5]. Essential oil of star anise has a sweetish, anise flavour and a highly aromatic odour. Due to its high level of phenolic volatile oils, star anise essential oil

could be used as a potential alternative antioxidant for synthetic antioxidant.

The objectives of this study were to extract essential oil from star anise using solvent extraction method, to determine the yields and antioxidant activities of essential oils at different extraction times and temperatures, to determine the quantity of trans-Anethole present in star anise essential oil using high performance liquid chromatography (HPLC), as well as tovalidate the presence of Anethole in star anise using Gas Chromatography–Mass Spectrometry (GC-MS).

MATERIALS AND METHOD

Collection of star anise

Star anise dried fruits were purchased from Tong Chun Tang traditional medicine store, Batang Kali, Selangor. The star anise fruits originated from Guangxi province, China. Star anise fruits were stored in plastic bags covered with newspapers to protect them from direct light. Finally, they were kept at room temperature.

Soaking of star anise

50g of dried star anise fruits were weighed using analytical balance (Model: ATX 224, Shimadzu, Japan). The whole star anise fruits were divided into separated carpels and were blended using blender (Model: MX-800S, Panasonic) for 20 seconds. The ground star anise was dissolved in 250 ml of absolute ethanol (ratio of crushed star anise to the ethanol was 1:5, w/v) for a given time (1, 3, 5, 7 and 9 days) at different temperatures (30, 40, 50, 60 and 70 °C).

Extraction of essential oil from star anise

This method was adapted from the work of Thuat and Ngoc (2010) with some modifications[6]. After the extraction time, the solution and solid part were separated by filtration using Whatman filter paper. The brown coloured filtrate was concentrated to volume of 100 ml using rotary evaporator (Model: Hei-vap Precision MLG3B, Germany) at 40 °C. The 100 ml concentrate was mixed with 100 ml of petroleum ether and 100 ml of dH_2O. The mixture was shook vigorously. After some time of settling, the petroleum ether solution (the upper layer) was dried over anhydrous Na_2SO_4. The petroleum ether solvent was removed using rotary evaporator to obtain essential oil. All the essential oils were stored in bijou glass bottles in dark condition at 4 °C before analysis.

Qualitative test using DPPH on TLC

This test was adapted from the work of Saleh and research group (2010) with a slight modification[7]. 10 µl of oil sample was spotted on a 10 x 10 cm silica glass plate. The plate was developed into 95:5, v/v toluene/ ethyl acetate as a mobile phase. After evaporation of the solvent, the plate was sprayed with 0.004 % solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The antioxidant activity was detected after 30 minutes. The yellow spots appeared from the reduction of DPPH was taken as positive results.

Quantitative test using DPPH scavenging assay

The antioxidant activity of star anise essential oil was measured in term of radical scavenging ability, using the stable free DPPH radical. This method was adapted from the work of Brand-Williams, Cuvelier and Berset (1995) with some modifications [8]. Stock solution (1mg/ml) was twofold diluted to different concentrations (0.0625, 0.125, 0.25, 0.5 and 1 mg/ml) with methanol. An aliquot of each dilution, about 1 ml was mixed with 1 ml of 0.004 % methanolic DPPH (0.004 g of DPPH in 100 ml of methanol). The mixture was vortexed and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm against a blank (1 ml of methanol with 1 ml of methanolic DPPH without the test oil) using spectrophotometer (Model: Genesys 20 4001/4). The radical scavenging activity or antioxidant activity (%) of each concentration of oil was calculated using the following formula:

$$\text{Antioxidant activity (\%)} = [\,(OD_{blank} - OD_{assay}) / (OD_{blank})\,] \times 100\ \% \quad \ldots(1)$$

The test was performed in triplicate. The antioxidant activities of the essential oils were expressed as EC_{50}, defined as the oil concentration in mg/ml required to scavenge 50 % of the DPPH free radical. BHT was used as positive control.

HPLC analysis

HPLC analysis was performed using Prominence, Shimadzu, Japan. The separation

column was Hypersil C18 column (250 mm x 4.6 mm, 5 µm). Essential oil was dissolved in acetonitrile (1 mg/ml). The sample was filtered using syringe with 0.45 µm filters prior to being filled into the vial. 5 ul of sample was injected into HPLC system and the injector temperature was 30°C. A gradient elution system using mobile phase of 15 %-65 % of acetonitrile with a flow rate of 1 ml/min for 45 minutes was used to identify the compounds present in the star anise essential oil.The detection wavelength was 275 nm.GC-MS analysis of the star anise sample was also done using GCMS 5977A from Agilent Technologies.

RESULTS AND DISCUSSION

The extraction yields of essential oils

Maximum yield of star anise essential oil (8.56 %) could be obtained by soaking star anise at 60 p C for 7 days (Table 1). In contrast, extraction of essential oil at temperature of 30 p C for 1 day gave minimum yield of essential oil (4.62 %). It was about 46.03 % less of extraction yield compared to the sample extracted at 60°C for 7 days.

There was contradictory report from previous researchers. From the previous study done by Ngoc (2006), dried star anise fruits have an essential oil content of 8-10 % [9]. The extraction yield of star anise essential oils in this study was 4.62-8.56 %, which was lower than that of extracted in previous study done by Ngoc. Different extraction yield of essential oil content might be due to several factors such as different geographical locations of the botanical materials, varying in cultivation condition, climate as well as post-harvest factors[10].

Besides, it was observed that the yield of essential oil increased slightly with increasing extraction time and temperature until a certain point. This result was in accordance with Dent and co-workers' (2012) study, reflected that yield increased with increasing extraction temperature and extraction time[11]. At temperature of 30°C, the extraction yield increased gradually with days. This result was in accordance with Kumar's previous study (2010), stated that the longer the extraction time, the longer the contact time of the plant material with the solvent and hence more yields of complete oil[12]. For temperature of 40°C and 70°C, the extraction yields decreased when star anise soaked for more than 5 days. Same behavior was observed at temperature of 50°C and 60°C, the yields decreased with an increase in extraction time at 9 days. This may be due to the loss of solvent by evaporation when extraction time and temperature increased simultaneously during extraction.

Table 1: Extraction yields at different extraction times and temperatures

Extraction	Extraction yields (%)				
time (days)	30°C	40°C	50°C	60°C	70°C
1	4.62	4.71	5.07	7.75	6.79
3	5.34	7.81	7.85	7.88	7.17
5	7.56	8.24	8.00	7.99	7.92
7	7.65	8.10	8.48	8.56	7.53
9	7.89	8.20	7.15	7.11	7.02

Table 2: Qualitative DPPH assay on TLC of the 25 samples studied

Extraction	Observation				
time (days)	30°C	40°C	50°C	60°C	70°C
1	+	+	+	+*	+*
3	+	+	+	+*	+*
5	+	+	+	+*	+*
7	+	+	+	+*	+*
9	+	+	+	+*	+*

The degree of activity was determined qualitatively from observation of the yellow intensity: weak (+) and strong (+).

Table 3: Number of antioxidant bands present in the star anise essential oils

Extraction	Number of antioxidant bands				
time (days)	30°C	40°C	50°C	60°C	70°C
1	3	3	3	3	3
3	3	2	3	3	3
5	2	3	3	3	3
7	3	3	3	3	3
9	3	3	2	2	3

Table 4: Antioxidant activity (%) of each concentration of star anise essential oils

Samples	Concentration (mg/ml)	AA* (%) ± SE
1 (30)	1.0000	50.65±0.59
	0.5000	36.14±0.89
	0.2500	24.79±1.38
	0.1250	14.94±1.64
	0.0625	9.35±1.97
3 (30)	1.0000	50.94±0.24
	0.5000	37.09±1.32
	0.2500	30.57±1.47
	0.1250	17.74±1.18
	0.0625	12.04±0.80
5 (30)	1.0000	50.92±0.10
	0.5000	42.55±0.36
	0.2500	33.78±0.28
	0.1250	20.40±0.21
	0.0625	7.57±0.36
7 (30)	1.0000	54.15±1.42
	0.5000	34.61±0.38
	0.2500	20.41±0.56
	0.1250	8.30±1.59
	0.0625	2.38±0.22
9 (30)	1.0000	58.29±1.00
	0.5000	46.95±1.37
	0.2500	42.24±1.49
	0.1250	27.84±1.32
	0.0625	21.53±0.98
1 (40)	1.0000	69.55±0.40
	0.5000	45.52±0.66
	0.2500	27.02±0.87
	0.1250	15.92±0.71
	0.0625	8.94±1.15
3 (40)	1.0000	73.50±1.17
	0.5000	58.34±0.36
	0.2500	33.84±0.84
	0.1250	17.01±0.97
	0.0625	11.76±1.50
5 (40)	1.0000	71.55±1.51
	0.5000	51.60±0.70
	0.2500	29.87±1.78
	0.1250	13.50±0.46
	0.0625	6.68±1.30
7 (40)	1.0000	65.35±0.96
	0.5000	48.74±0.65
	0.2500	42.20±0.32
	0.1250	33.30±0.73
	0.0625	27.85±1.28
9 (40)	1.0000	72.70±1.22
	0.5000	45.22±0.78
	0.2500	23.16±1.12
	0.1250	18.00±0.35
	0.0625	8.00±0.49
1 (50)	1.0000	75.54±0.57
	0.5000	69.88±1.08
	0.2500	69.47±0.52
	0.1250	55.87±0.47
	0.0625	39.76±1.24
3 (50)	1.0000	76.58±0.66
	0.5000	74.49±0.62
	0.2500	61.99±1.05
	0.1250	53.16±1.99
	0.0625	34.53±0.80
5 (50)	1.0000	75.26±0.88
	0.5000	69.07±0.73
	0.2500	65.58±0.44
	0.1250	48.46±1.23
	0.0625	35.79±1.10
7 (50)	1.0000	72.51±0.31
	0.5000	68.67±1.83
	0.2500	66.27±0.93
	0.1250	46.54±1.13
	0.0625	32.72±1.55
9 (50)	1.0000	77.43±0.97
	0.5000	76.73±1.73
	0.2500	73.34±0.49
	0.1250	57.16±1.07
	0.0625	35.19±0.82
1 (60)	1.0000	77.76±0.15
	0.5000	67.10±1.03
	0.2500	62.62±0.74
	0.1250	57.76±1.16
	0.0625	42.25±1.17
3 (60)	1.0000	76.71±0.68
	0.5000	72.26±1.44
	0.2500	53.81±1.13
	0.1250	30.61±0.56
	0.0625	16.03±1.22
5 (60)	1.0000	77.70±0.60
	0.5000	72.30±0.27
	0.2500	67.50±0.50
	0.1250	41.78±1.69
	0.0625	25.72±1.41
7 (60)	1.0000	72.87±0.25
	0.5000	72.44±0.61
	0.2500	61.35±0.19
	0.1250	39.84±1.17
	0.0625	30.46±1.31

9 (60)	1.0000	71.50±0.52
	0.5000	68.92±0.78
	0.2500	58.52±1.47
	0.1250	42.37±0.19
	0.0625	25.69±0.96
1 (70)	1.0000	76.76±0.58
	0.5000	74.20±0.38
	0.2500	70.52±0.94
	0.1250	47.72±0.55
	0.0625	27.86±1.49
3 (70)	1.0000	72.71±0.89
	0.5000	69.38±0.34
	0.2500	62.73±0.64
	0.1250	55.99±0.54
	0.0625	35.25±1.22
5 (70)	1.0000	74.36±0.67
	0.5000	70.35±0.66
	0.2500	68.89±0.77
	0.1250	52.39±0.52
	0.0625	33.42±1.50
7 (70)	1.0000	71.53±0.97
	0.5000	68.27±0.61
	0.2500	63.62±0.98
	0.1250	48.78±0.71
	0.0625	33.53±1.40
9 (70)	1.0000	75.28±1.63
	0.5000	65.81±1.33
	0.2500	43.75±1.66
	0.1250	28.69±0.77
	0.0625	21.10±1.77
Positive control		
BHT	0.0500	77.93±0.85
	0.0250	63.89±0.95
	0.0125	43.79±1.40
	$6.25x10^{-3}$	35.54±0.63
	$3.125x10^{-3}$	22.28±1.12

*1, 3, 5, 7 and 9 are the extraction time (days)while (30), (40), (50), (60) and (70) are temperature (p C).

* AA is antioxidant activity (%).

For star anise soaked for 1, 3 and 7 days, the yield of essential oil increased from 30°C to 60°C. The extraction yield decreased with a further increase in temperature at 70°C due to loss of solvent and volatile oil at high temperature. The increase of extraction yield with increasing extraction time and temperature may be due to increased solubility and diffusion coefficient[11]. In this present study, the extraction temperature of 60°C and the extraction time of 7 days were the best condition to increase the extraction efficiency of star anise essential oil. It is therefore suggested that an extraction temperature of no higher than 60°C is employed.

Qualitative test using DPPH on TLC

All the star anise essential oils extracted at varied extraction times and temperatures showed antioxidant activities (Table 2). The degree of antioxidant activity of all samples was determined qualitatively from observation of the yellow colour intensity. Based on the yellow intensity of the bands on the chromatograms, the essential oil from star anise extracted at 60°C and 70°C for 1 day, 3 days, 5 days, 7 days and 9 days apparently showed stronger antioxidant activity than those extracted at 30°C, 40°C and 50°C.

Besides, it was observed that at least two different bands with antioxidant activity with different polarity were appeared on the TLC plate after spraying with DPPH and incubated for 30 minutes. (Figure 1-5). Samples extracted at 30°C for five days, 40°C for three days, 50° C and 60°C for nine days had two active bands while the rest of the samples showed three active bands with antioxidant activity (Table 3). The R_f values of the active bands in all samples were in the range of 0.41 to 0.65.

Quantitative test using DPPH scavenging assay

The radical scavenging activity or antioxidant activity (%) of each concentration of oil and BHT were calculated using the formula (Equation 1) and the results were shown in Table 4. Generally, all the samples extracted at 303.3°C for 1, 3, 5, 7 and 9 days showed low antioxidant activity, which was below 60 %. Figure 6 showed that the antioxidant activity (%) of essential oils extracted at 30°C increased steadily with increasing concentration from 6.25 x 10^{-3} mg/ml to 1.00 mg/ml. The antioxidant activity of each sample was then expressed as EC_{50}, which is the concentration of sample required to decrease the initial DPPH free radical by 50 %.

It was observed that star anise essential oil extracted at 30°C for 1 day had the greatest EC_{50} value of 0.973 mg/ml (Table 5). This means that the essential oil extracted at 30°C for 1 day had lowest antioxidant activity as it required 0.973 mg/ml to scavenge 50 % of the free DPPH radicals.

The EC_{50} values for samples extracted at 30°C for 3 days (0.967 mg/ml), 5 days (0.928 mg/ml), 7 days (0.893 mg/ml) and 9 days (0.622 mg/ml) were declined gradually. This indicated that the degree of antioxidant activity increased with increasing extraction time at 30°C. This might be due to longer contact time of star anise with solvent and hence increased the rate of extraction of potential active compounds with antioxidant activity[12]. The ascending order for the antioxidant activity was 1 (30° C) < 3 (30°C) < 5 (30°C) < 7 (30°C) < 9 (30°C).

The antioxidant activity (%) of all the star anise essential oil extracted at 40°C was above 60 % (Table 4 and Figure 7). The EC_{50} value for essential oil extracted at 40°C for 1 day was 0.585 mg/ml (Table

Table 5: EC_{50} of star anise essential oils extracted at 30°C for 1, 3, 5, 7 and 9 days

Samples	EC_{50} (mg/ml)
1 (30°C)	0.973 ± 0.56
3 (30°C)	0.967 ± 0.56
5 (30°C)	0.928 ± 0.54
7 (30°C)	0.893 ± 0.52
9 (30°C)	0.622 ± 0.36

*EC_{50} defined as the concentration of essential oil in mg/ml required to scavenge 50% of the DPPH radical

Table 6: EC_{50} of star anise essential oils extracted at 40°C for 1, 3, 5, 7 and 9 day

Samples	EC_{50} (mg/ml)
1 (40°C)	0.585 ± 0.34
3 (40°C)	0.391 ± 0.23
5 (40°C)	0.476 ± 0.27
7 (40°C)	0.528 ± 0.30
9 (40°C)	0.583 ± 0.34

*EC_{50} defined as the concentration of essential oil in mg/ml required to scavenge 50% of the DPPH radical

Table 7: EC_{50} of star anise essential oils extracted at 50°C for 1, 3, 5, 7 and 9 days

Samples	EC_{50} (mg/ml)
1 (50°C)	0.098 ± 0.06
3 (50°C)	0.112 ± 0.06
5 (50°C)	0.133 ± 0.08
7 (50°C)	0.143 ± 0.08
9 (50°C)	0.099 ± 0.06

*EC_{50} defined as the concentration of essential oil in mg/ml required to scavenge 50% of the DPPH radical

Table 8: EC_{50} of star anise essential oils extracted at 60°C for 1, 3, 5, 7 and 9 days

Samples	EC_{50} (mg/ml)
1 (60°C)	0.089 ± 0.05
3 (60°C)	0.221 ± 0.13
5 (60°C)	0.153 ± 0.09
7 (60°C)	0.173 ± 0.10
9 (60°C)	0.583 ± 0.34

*EC_{50} defined as the concentration of essential oil in mg/ml required to scavenge 50% of the DPPH radical

Table 9: EC_{50} of star anise essential oils extracted at 70°C for 1, 3, 5, 7 and 9 days

Samples	EC_{50} (mg/ml)
1 (70°C)	0.135 ± 0.08
3 (70°C)	0.100 ± 0.06
5 (70°C)	0.116 ± 0.07
7 (70°C)	0.130 ± 0.08
9 (70°C)	0.310 ± 0.18

*EC_{50} defined as the concentration of essential oil in mg/ml required to scavenge 50% of the DPPH radical

Table 10: EC_{50} of BHT

Positive control	EC_{50} (mg/ml)
BHT	0.016 ± 0.01

*EC_{50} defined as the concentration of BHT in mg/ml required to scavenge 50% of the DPPH radical

6). The EC_{50} value dropped to 0.391 mg/ml when the essential oil extracted at 40°C for 3 days. After that, there was an increase in EC_{50} values until day 9. The increase of EC_{50} values corresponded to a weaker

Table 11: Retention time, peak area, area percentage and concentration (%) of trans-Anethole

Samples	Retention time (min)	Peak area (mAU)	Area %	Concentration of trans-Anethole (%)
1 (30°C)	36.447	42026575	87.10	45.03
3 (30°C)	36.494	44773802	88.65	47.77
5 (30°C)	35.969	51472038	86.10	54.47
7 (30°C)	35.892	47123286	83.59	50.12
9 (30°C)	35.953	45643690	88.22	48.64
1 (40°C)	36.159	74506751	90.59	77.51
3 (40°C)	35.643	77681832	83.98	80.68
5 (40°C)	35.416	77231780	81.75	80.23
7 (40°C)	35.688	75850066	83.65	78.85
9 (40°C)	35.480	72687388	81.03	75.69
1 (50°C)	36.085	87166545		90.17
3 (50°C)	35.674	86293969	91.87	89.29
5 (50°C)	35.515	85001535	82.86	88.00
7 (50°C)	35.695	84310937	79.95	87.31
9 (50°C)	35.501	87883643	80.82	90.88
1 (60°C)	35.593	88605132	77.66	91.61
3 (60°C)	35.602	80384982	81.01	83.38
5 (60°C)	36.635	83499402	81.49	86.50
7 (60°C)	36.387	81248763	77.29	84.25
9 (60°C)	36.217	82197107	85.26	85.20
1 (70°C)	35.984	84675286	84.13	87.68
3 (70°C)	35.777	86826860	78.16	89.83
5 (70°C)	35.505	86137109	80.11	89.14
7 (70°C)	36.143	84713397	81.17	87.71
9 (70°C)	35.960	80227656	80.90	83.23

Table 12: GC-MS integration peak list of star anise

Peak	Start	Retention Time (min)	End	Height	Area	Area %
1	1.207	1.254	1.278	12317328.01	20705118.73	26.51
2	1.278	1.296	1.320	1007344.37	1259429.21	1.61
3	1.320	1.333	1.405	1399943.10	1631685.47	2.09
4	1.471	1.490	1.508	872502.26	846001.88	1.08
5	1.580	1.598	1.677	2163750.47	2163120.54	2.77
6	6.921	6.982	7.018	4293290.40	9256418.18	11.85
7	7.018	7.042	7.109	759609.95	1450076.10	1.86
8	8.704	8.752	8.855	1110739.09	2250361.35	2.88
9	11.351	11.411	11.495	2060092.29	4496388.18	5.76
10	13.672	13.84	13.912	15702253.33	78099815.51	100.00

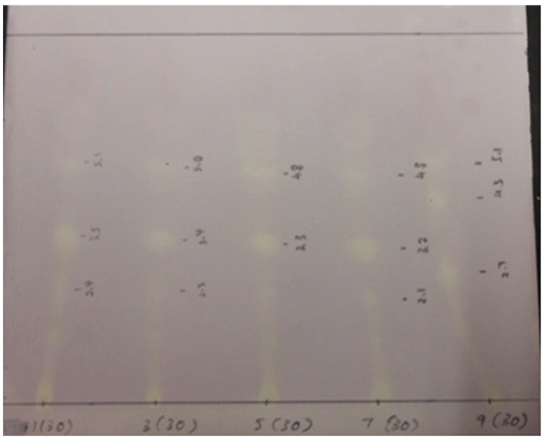

Fig. 1: Chromatogram of essential oil extracted at 30°C for 1 day, 3 days, 5 days, 7 days and 9 days (from left to right)

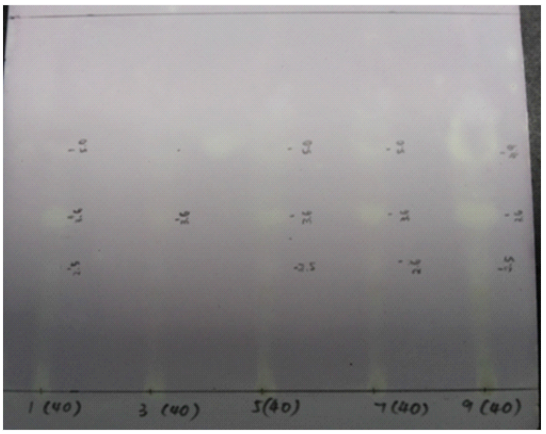

Fig. 2: Chromatogram of essential oil extracted at 40°C for 1 day, 3 days, 5 days, 7 days and 9 days (from left to right)

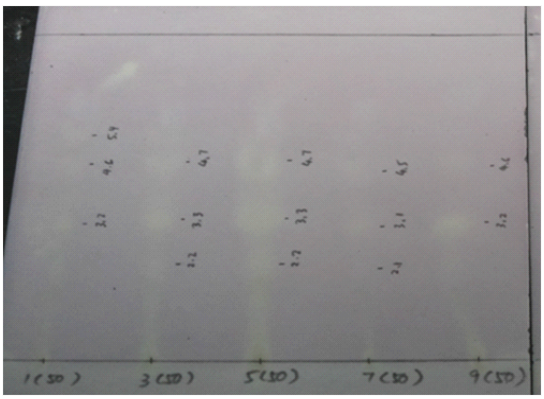

Fig. 3: Chromatogram of essential oil extracted at 50°C for 1 day, 3 days, 5 days, 7 days and 9 days (from left to right)

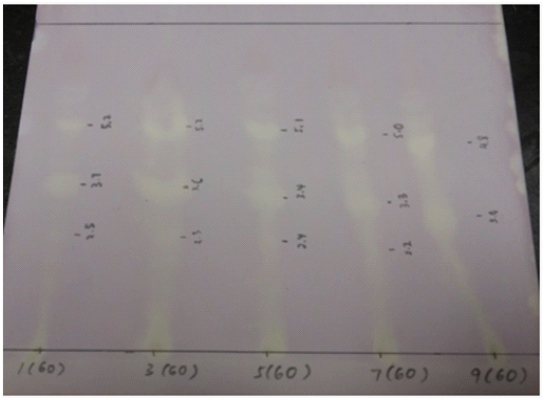

Fig. 4: Chromatogram of essential oil extracted at 60°C for 1 day, 3 days, 5 days, 7 days and 9 days (from left to right)

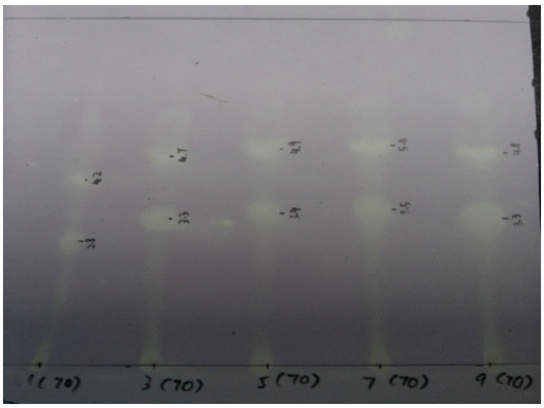

Fig. 5: Chromatogram of essential oil extracted at 70°C for 1 day, 3 days, 5 days, 7 daysand 9 days (from left to right)

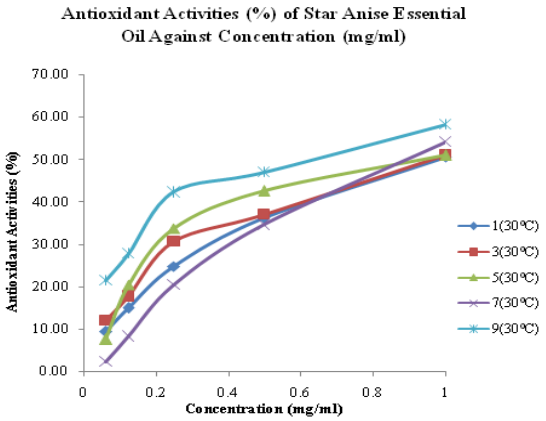

Fig. 6: DPPH radical scavenging activity of star anise essential oils extracted at 30°C for 1,3, 5, 7 and 9 days

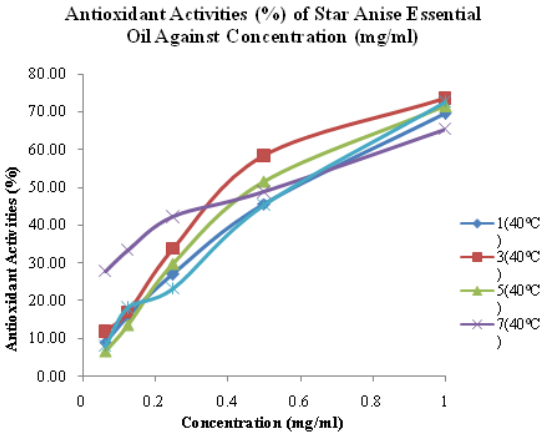

Fig. 7: DPPH radical scavenging activity of star anise essential oils extracted at 40°C for 1, 3, 5, 7 and 9 days

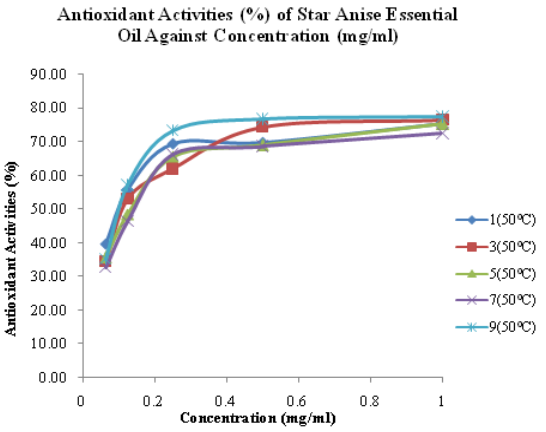

Fig. 8: DPPH radical scavenging activity of star anise essential oils extracted at 50°C for 1,3, 5, 7 and 9 days

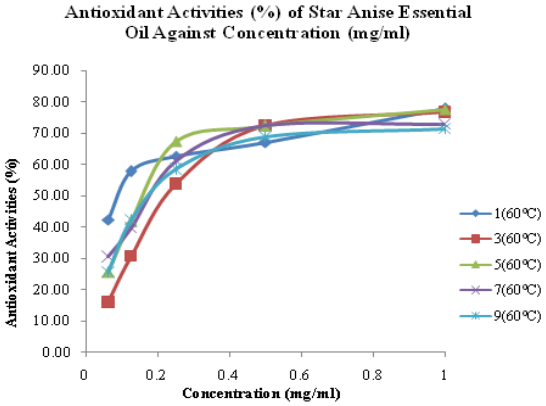

Fig. 9: DPPH radical scavenging activity of star anise essential oils extracted at 60 °C for 1, 3, 5, 7 and 9 days.

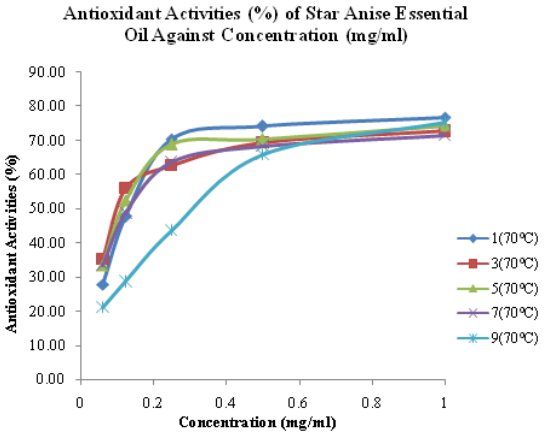

Fig. 10: DPPH radical scavenging activity of star anise essential oils extracted at 70°C for 1, 3, 5, 7 and 9 days

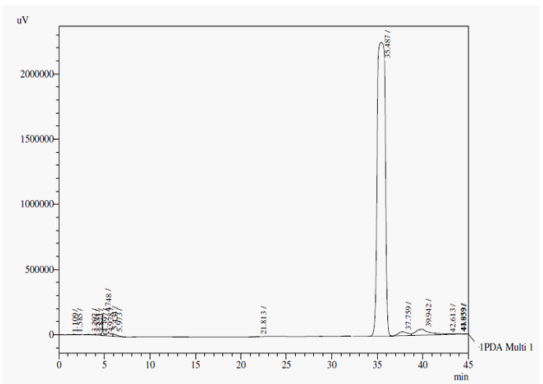

Fig. 11: HPLC chromatogram for standard trans-Anethole

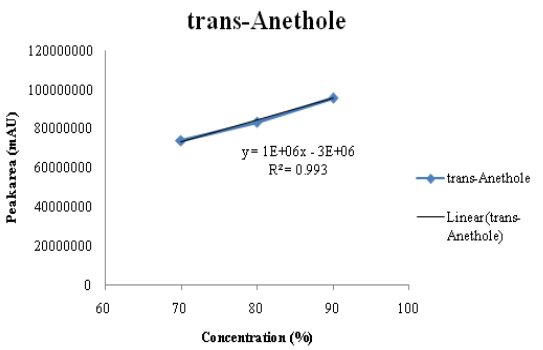

Fig. 12: Standard curve of trans-Anethole

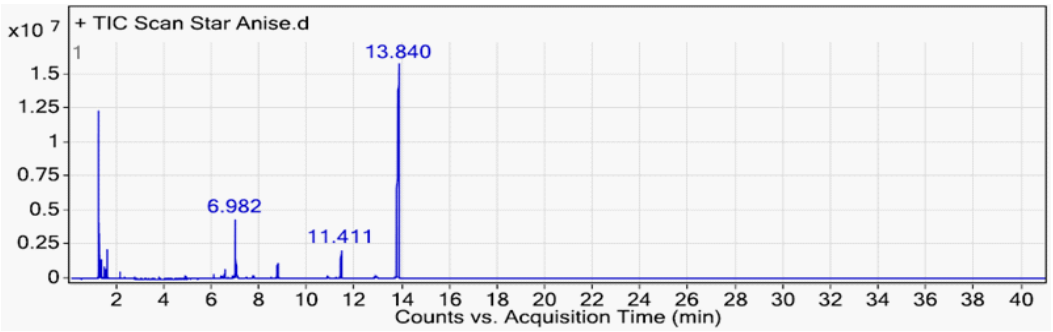

Fig. 13: GC-MS chromatogram of Star Anise

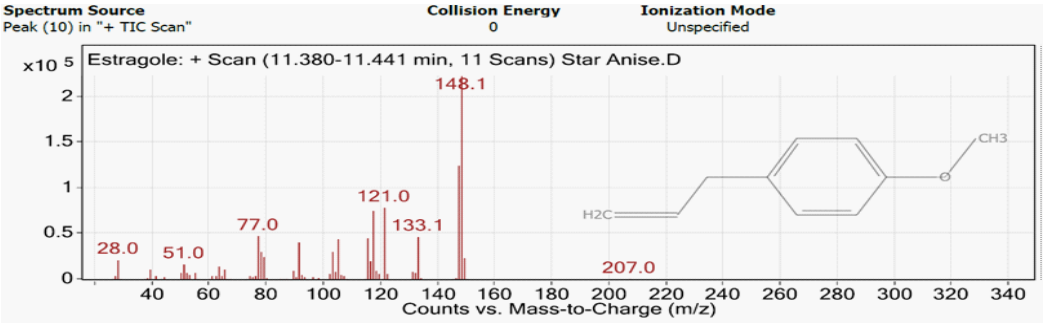

Fig. 14: GC-MS spectrum source of Estragole at peak 10

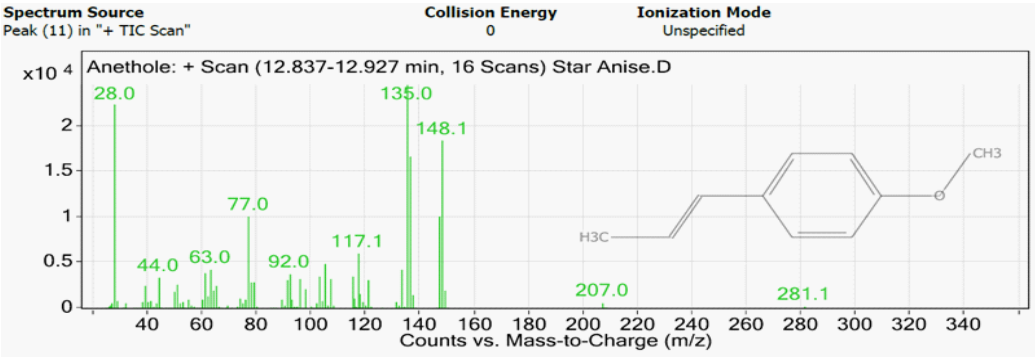

Fig. 15: GC-MS spectrum source of Anethole at peak 11

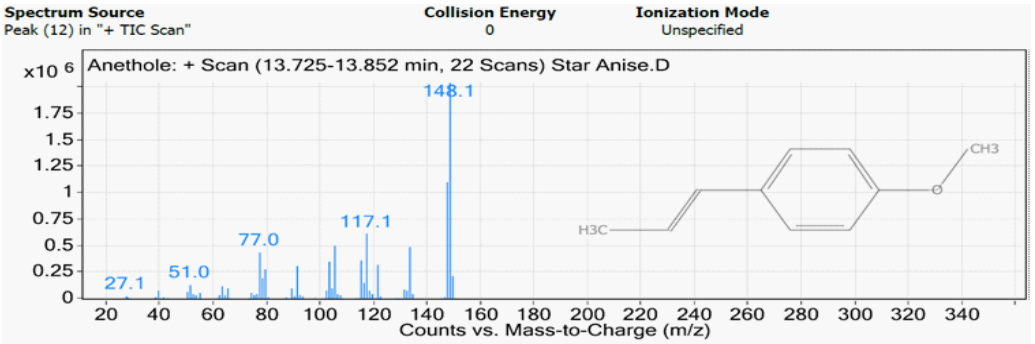

Fig. 16: GC-MS spectrum source of Anethole at peak 12

antioxidant.The order of antioxidant capacity for samples extracted at 40°C was 1 (40°C) < 9 (40°C) < 7 (40°C) < 5 (40°C) < 3 (40°C).

In general, the antioxidant activity (%) of star anise essential oils extracted at temperature of 50° C was higher than 70 % (Table 4 and Figure 8). According to Table 7, essential oil extracted at 50 p C for 1 day showed EC_{50} value of 0.098 ± 0.06 mg/ml. The concentration of essential oil needed to scavenge 50 % of DPPH radical increased from day 1 to day 7. However, there was a marked decrease of concentration of essential oil to scavenge 50 % of the radicals. The antioxidant power of sample extracted at 50°C for 9 days (0.099 ± 0.06 mg/ml) was as good as the sample extracted for 1 day. In other words, essential oil extracted at 50°C for 1 day was the strongest antioxidant, followed by those essentail oil extracted at 9 days, 3 days, 5 days and 7 days.

All the essential oils extracted at 60°C showed antioxidant activity greater than 70 % (Table 4 and Figure 9). The results in Table 8 showed that essential oils extracted at 60°C for 1 day was the strongest antioxidant. This was because only 0.089 ± 0.05 mg/ml of essential oil was needed for the scavenging activity of free radicals. The antioxidant power was decreasing in the order 1(60° C) > 5(60°C) > 7(60°C) > 3 (60°C) > 9 (60°C).

Compared with essential oil extracted at 60 p C for 1 day (EC_{50} value= 0.089 ± 0.05), essential oil extracted at 60 p C for 9 days (EC_{50} value= 0.583 ± 0.34) showed a significant difference in term of concentration in mg/ml necessary to scavenge 50 % of the DPPH radicals. The antioxidant activities of star anise essential oils extracted at 60° C were undulated with increasing extraction time. This might be due to the possible synergistics effects of different compounds on the total oil antioxidant activity[13].

All the star anise essential oils extracted at 70°C for varied extraction time showed antioxidant activity (%) above 70 % (Table 4 and Figure 10). Since lower EC_{50} value indicated higher antioxidant activity, essential oil extracted at 70 p C for 3 days exhibited stronger antioxidant activity than essential oil extracted for 1 day. The EC_{50} value for essential oil extracted for 3 days and 1 day were 0.100 ± 0.06 mg/ml and 0.135 ± 0.08 mg/ml respectively as shown in Table 9. Essential oils extracted more than 3 days, which were 5 days, 7 days and 9 days showed decreasing of antioxidant power as the EC_{50} value increased markedly from 0.100 ± 0.06 mg/ml to 0.310 ± 0.18 mg/ml. The antioxidant activity was in order 3 (70°C) > 5 (70°C) > 7 (70° C) > 1 (70° C) > 9 (70°C).

There was an increase in antioxidant activities of star anise essential oils from 30°C to 50° C. This might due to ambient or physiological temperatures could reduce thermal degradation of volatile essential oil[14]. The antioxidant activities of star anise essential oils decreased slightly at 60°C. This was in agreement with Dent and research group (2012), stated that degradation of phenolic compounds may occured at temperature of 60° C[11]. At temperature 70°C, the antioxidant activities of star anise essential oils increased moderately. Typically, antioxidant activity decreased with increasing temperature, but it does not have universal validity[15].

In this quatitative antioxidant test using DPPH method, a well known synthetic antioxidant, namely butylated hydroxytoluene (BHT) was used as positive control. The concentration of BHT for a 50 % scavenging activity was about 0.018 mg/ml [16]. In this study, 0.016 ± 0.01 mg/ml of BHT was required in scavenging action towards DPPH free radicals (Table 10).By comparing the antioxidant activity of star anise essential oil with BHT, it was revealed that antioxidant activity of essential oil from star anise was comparable with the synthetic antioxidant BHT, particularly essential oils that were extracted at temperature 50°C and 60°C.

HPLC and GC-MS analysis

The HPLC chromatogram of standard trans-Anethole with retention time of 35.487 minutes was shown in Figure 11. It was observed that the highest peaks of all samples have a retention time near to the retention time of standard trans-Anethole, which was around ±35 minutes. Similarities of the highest peak and retention time between samples and standard trans-Anethole in the HPLC chromatogram confirmed that trans-Anethole was present in star anise essential oil. In order to identify the concentration (%) of trans-Anethole present in all the essential oils, a standard curve of trans-Anethole was plotted (Figure 12). The retention times, peak areas, area

percentage and concentration (%) of trans-Anethole for all samples were shown in Table 11.

All the star anise essential oils had high area percentage of trans-Anethole, which was ranged from 77.29 % to 91.87 %. High percentage area indicated trans-Anethole was the most abundant compounds in all the star anise essential oils. Similar results were reported previously by Chempakam and Balaji (2008), stated that the main component of star anise essential oil is trans-Aanethole, which accounts for 80-90 %[4]. From GC-MS analysis, Estragole was found to have the most significant peak with peak area 100% at retention time 13.840 minutes as shown in Figure 13 and 14 and Table 12.Estragole was found at the tenth peak. Anethole was also found at two peaks which were peak 11 and 12 as shown in Figure 15 and Figure 16. Thus, the data validates the presence of Anethole in the star anise sample.

In addition, it was observed that the concentration (%) of trans-Anethole in the star anise essential oils varied considerably as function of temperature and extraction time. Generally, star anise essential oils extracted at 40°C, 50°C, 60°C and 70°C contained relatively high concentration (%) of trans-Anethole compared to essential oils extracted at 30°C. As temperature increases, higher mass fraction of trans-Anethole was extracted. It was in agreement with Chen and co-workers' finding (2007), which concluded that higher temperature will reduce the solvent viscosity and facilitate the diffusion of molecules, resulting to an increase of extraction efficiency[17].

Besides, it was found that the concentration (%) of trans-Anethole corresponded to the antioxidant activities of star anise essential oils. This statement was in agreement with Padmashree and research group (2007), reported that the antioxidant activity is due to high percentage of trans-Anethole, which is more than 80 % [18]. In the present study, star anise essential oil extracted at 60°C for 1 day has the highest concentration of trans-Anethole (91.61 %) and antioxidant activity (EC_{50}=0.089 mg/ml) while star anise essential oil extracted at 30°C for 1 day has the lowest concentration of trans-Anethole (45.03 %) and antioxidant activity (EC_{50}=0.973 mg/ml). Therefore, it might be possible to infer that trans-Anethole found in the samples contributed to the antioxidant activities of the essential oils.

CONCLUSION

Essential oil from star anise was successfully extracted using solvent extraction method. Different extraction times (1 day, 3 days, 5 days, 7 days and 9 days) and temperatures (30°C, 40°C, 50°C, 604.0° C and 70°C)did affect the yield of essential oil. The best condition to obtain the highest yield (8.56 %) of essential oil was at temperature of 60°C with an extraction time of 7 days. A rapid screening using DPPH assay on TLC showed thatat least two different yellow spots with antioxidant activity with different polarity were appeared on all TLC plates at solvent system 95:5, v/v toluene/ ethyl acetate. In addition, the highest antioxidant activity of star anise essential oil was observed when the sample was extracted at 60°C for 1 day (EC_{50} value = 0.089±0.05 mg/ml).From the HPLC analysis results, samples with higher trans-Anethole concentration have higher antioxidant activities. Star anise essential oil extracted at 60° C for 1 day showed the highest concentration (%) of trans-Anethole (91.61 %) while essential oil extracted at 30°C for 1 day showed the lowest concentration (%) of trans-Anethole (45.03 %). The antioxidant activity of the high level of trans-Anethole-containing star anise essential oil was comparable with the synthetic antioxidant BHT.

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