Available Online at ESci Journals
Journal of Food Chemistry and Nutrition
ISSN: 2307-4124 (Online), 2308-7943 (Print)
http://www.escijournals.net/JFCN

EXTRACTION OF ESSENTIAL OIL FROM *CURCUMA LONGA*

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A B S T R A C T

Turmeric or its scientific name *Curcuma Longa* has been used for preparing traditional Indian curries for hundreds of years as a flavor, color and preservative. Turmeric has been reported to possess anti-inflammatory, hepatoprotective, antitumor, antiviral activities and anticancer activity and is used in gastrointestinal and respiratory disorders. In this study, a solvent extraction method has been used to extract active compound which was curcumin in *Curcuma Longa.* Ethanol has been used as solvent to extract the curcumin. Rotary evaporator was used to purify the sample. The samples were put in rotovap at temperatures 40°C, 50°C, 60°C and 70°C. HPLC analysis showed that all of the samples had high percentage of curcumin. The antioxidant activity in 50°C curcumin extract showed the highest antioxidant activity with 24.968 M IC_{50} value while the lowest was the 70°C curcumin extract with 111.93 M IC_{50} value. The higher the IC_{50} value, the lower the antioxidant activity.

Keywords: turmeric, solvent extraction, essential oil, curcumin, antioxidant activity.

INTRODUCTION

Medicinal plants have been used for mankind as source of medicines since a long time ago. More than 35,000 plant species have been reported to be used in various human cultures around the world for medical purposes. Plant oils and extracts have been used for a wide variety of purposes for thousands of years. One of their purposes is as a source of medicine as they contain organic compounds with therapeutic values. Majority of people depend on traditional medicine as their primary healthcare. About 80% of people in this world depend on herbs for health. In most developing countries, the use of medicinal plants which is a basis for maintenance of good health has been discovered (Tsai *et al.,* 2011).

Besides serving medicinal purposes, the plant extracts are also used as herbs and spices. These spices and herbs are considered safe and effective against certain ailments. A long-term consumption of these substances also guaranteed not to cause any side effects. Herbs such as ginger, turmeric and galanga have been used as medicine in traditional Indian medicine or Ayurveda. Ayurvedic medical systems have different uses for both fresh and dried preparations. The dried powders are used to treat distinctly different ailments from pastes or plant juices (Cousins *et al.,* 2007). Herbs are also used traditionally in Ayurvedic medicine as antiseptic, wound healing, and anti-inflammatory compounds (Chang *et al.,* 2006).

Zingiberaceae family comprises of many genera of aromatic and medicinal plants such as Curcuma, Alpinia, Zingiber and Kaempferia and one of the plants that commonly found in Malaysia is *Curcuma Longa Linnaeus* or turmeric. *Curcuma Longa* is sometimes called *Curcuma domestica* as it is always used in the kitchen for preparing dishes. *Curcuma Longa* has been used for preparing traditional Indian curries for hundreds of years as a flavor, color, and preservative (Tsai *et al.,* 2011).

Commercially, it is traded as dye, spice, and source of industrial starch. India produces about 400,000 tons fresh weight per year or about 80% of the world's supply of commercial turmeric (Cousins *et al.,* 2007). The yellow color, which is characteristic of the turmeric rhizome, is due to the presence of 3–5% of curcuminoids. The curcuminoids includes curcumin, demethoxycurcumin, bisdemethoxycurcumin and cyclocurcumin of which curcumin is the major bio-active

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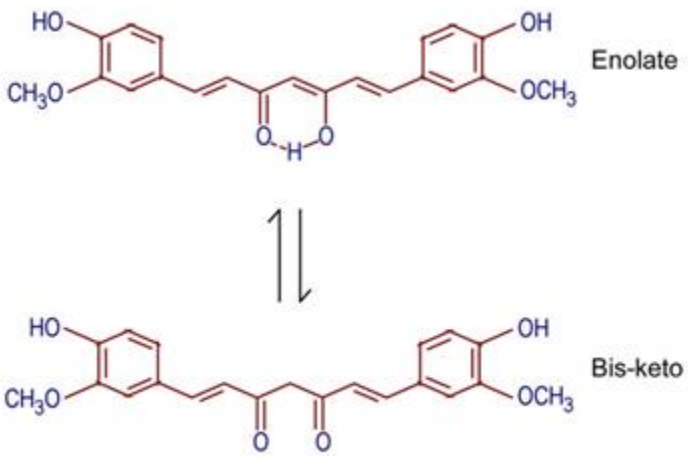
constituent (Wakte *et al.,* 2011). Figure 1 showed turmeric plant with rhizome and curcumin chemical structures.

Many studies have reported the biological, physiological and chemical properties of turmeric. Turmeric has been reported to possess anti-inflammatory, hepatoprotective, antitumor, antiviral activities and anticancer activity and is used in gastrointestinal and respiratory disorders. Curcuminoids exhibit free-radical scavenging properties, antioxidant activity and act as inhibitors of human immune deficiency virus type 1 (HIV-1) integrase. The main biological activities of the oil are carminative, antiflatulence, antifungal and as an antiplatelet agent. The Thai Herbal Pharmacopoeia [THP] (1995) recommended that dried turmeric should contain not less than 6.0% v/w of turmeric oil and 5.0% w/w of total curcuminoids (THP, 1995). Several ginger plants have been reported to produce interesting metabolites with interesting bioactivities such as antimicrobial, antioxidant, anti-inflammatory and anti-tumor promoters. The chemical constituents in essential oils and crude extracts of Zingiberaceae plants have been reported to have these properties which have effects on living organisms. In this study, *Curcuma longa* was chosen to be studied and analysed for its antioxidant activity. Ethanol was used as the solvent to extract the essential oil.

Turmeric plant with Rhizome



Curcumin

Figure 1. TOC graphic figure of Tumeric plant with rhizome and Curcumin chemical structures.

The objectives of the research were to extract the essential oil (curcumin) of *Curcuma Longa* using solvent extraction, to identify curcumin compound using High Performance Liquid Chromatography (HPLC) and to determine the antioxidant activity of *Curcuma Longa*. This research emphasizes on the study of chemical compounds and antioxidant activities of *Curcuma longa* from Malaysia.

MATERIALS AND METHODS

Plant Material: The fresh rhizomes of *C. longa* were purchased from the wet market. The rhizomes were chopped into small pieces, air-dried and then extracted using solvent extraction.

Solvent Extraction: Fresh rhizomes were washed with water, sliced and sun dried for a week and dried at 50°C in hot air oven for 6 hours. Dried rhizomes were cut in small pieces. Samples weighing 500g were transferred carefully into a glass bottle and 500 liter of 100% ethanol was added. The bottle was gradually shaken to mix up the materials and placed in the dark at room temperature.

Extract purification: The extract sample was filtrated by using Muslin clothes followed by Whitman No.1 filter paper, the clear solution was collected in an amber reagent bottle. This process helped to remove the powder plant but the filtrate was not pure enough since it still contained the impurities and ethanol. In order to obtain high concentration of the sample, further process had to be done by using rotary evaporator (Revathy, 2011). The bath temperatures were set at 40°C, 50°C, 60°C and 70°C and the rotation at 90rpm. The final product was collected and transferred into glass bottle

and ready to be further analyzed using High Performance Liquid Chromatography (HPLC), DPPH free radical scavenging assay and UV spectrophotometer. The HPLC analysis was determined as described by Kamble *et al.* (2011).

High Performance Liquid Chromatography (HPLC): The active compounds of *Curcuma Longa* were identified using HPLC method.

i) Preparation of sample*:* 25 mg of sample was dissolved in 25 ml ethanol. 1 ml of the sample was then diluted to 5 ml with ethanol and filtered through 0.2µm membrane filter before injection.

ii) ii) Chromatographic conditions*:* Samples were analyzed in Shimadzer LC 20A0 liquid chromatograph system with SPD-M20AuV detector in isocratic mode. The elution was carried out with gradient solvent system with flow rate 1 ml/min at 40°C temperature. The mobile phase consisted of methanol (23%), acetonitrile (41%) water (35%) and acetic acid (1%) (v/v) basis. The sample was injected at 20 µl. An HP 2000 series ultra-violet detector was used at wavelength 425 nm for detection of curcumin.

Tests for antioxidant activity: In this study, DPPH free radical scavenging assay and UV spectrophotometer were used to analyze the results.

Stock solutions of sample extracts were prepared at 0.1 mg/ml concentration in 100% methanol. The samples were serially divided into five concentrations which were 0.02, 0.04, 0.06, 0.08 and 0.10 mg/ml and placed in test tubes. DPPH was also prepared with five different concentrations as the samples. DPPH was prepared in methanol at 0.002% and 1000µl of the solution was mixed with each sample concentrations and standard solution separately.

These solution mixtures were kept in the dark for 30 minutes and the optical densities were measured at 517 nm using a UV spectrophotometer (Genesys 20). Methanol (1000 µl) mixed with DPPH solution (0.002% and 1000 µl) was used as a blank. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were used as a standard.

All the tests were performed in triplicate and the graph was plotted with the average of three observations (Kumaran and Karunakaran, 2007). The optical density was recorded and the percentage of total radical scavenging activity was calculated using the formula given in Equation 1:

$$\text{DPPH activity \%age} = \left[\frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}}\right] \times 100$$

where A is the absorbance.

Statistical analysis: The antiradical activity was expressed as IC_{50} (µg/mL), the antiradical dose required to cause 50% inhibition. A lower IC_{50} value corresponds to a higher antioxidant activity of plant extract. T-test analysis was used to compare the IC_{50} of the samples with ascorbic acid (standard) because they have different final concentrations. T-test analysis was also used to compare the mean inhibition of radical scavenging activity between the samples. If the P value was $p \leq 0.01$, the data was significantly different. On the other hand, if the P value was $p \geq 0.01$, the data was not significantly different.

RESULTS AND DISCUSSION

Effect of temperature on time needed for curcumin extraction: Figure 2 showed the time needed for curcumin extraction at different temperatures (40°C 50°C, 60°C and 70°C). The experiment was carried out at fixed volume of turmeric extract which was 100 ml, with constant speed of rotation at 90 rpm. Fastest extraction was obtained when temperature was at 70°C which was 25 minutes. Longest time needed for extraction of curcumin was at lower temperature, 40°C which took 90 minutes. Other solvent extraction methods using acetone and hexane were also done by Kamble *et al.* (2011). Curcumin extraction by solvent ethanol and acetone were statistically at par. Hexane was found to be the weakest amongst the solvents tested (Kamble *et al.,* 2011). According to Paulucci *et al.* (2013), the optimum temperature for curcumin extraction was found to be at 80°C, along with 12 h of extraction time and agitation speed of 30 rpm. Meanwhile in a study done by Sogi *et al.* (2010), the temperature of 60°C and mixing time of 30 min using ethanol as the solvent were found to yield maximum curcumin. Factors such as the extraction method, the solvents used for extraction, the extraction time and temperature, the solvent ratio and extraction pressure were among the significant factors that were shown to be able to influence the efficiency of curcumin extraction (Wakte *et al.,* 2011).

Curcumin standard analysis: The elution was carried out with gradient solvent system at flow rate 1 ml/min at temperature 40°C (Kamble *et al.,* 2011). The column used was Hypersil BDS C18 column 250mm x 4.6mm, 5 µm particle size. Table 1 showed the summary of the conditions used for HPLC analysis.

Table 1: Conditions of HPLC analysis

Analysis:	Curcumin in ethanol extract	Time	: 10 minutes
Column:	Hypersil C18		
Injection volume:	20µl		
Mobile phase:	methanol (23%), acetonitrile (41%) water (35%) and acetic acid (1%)		

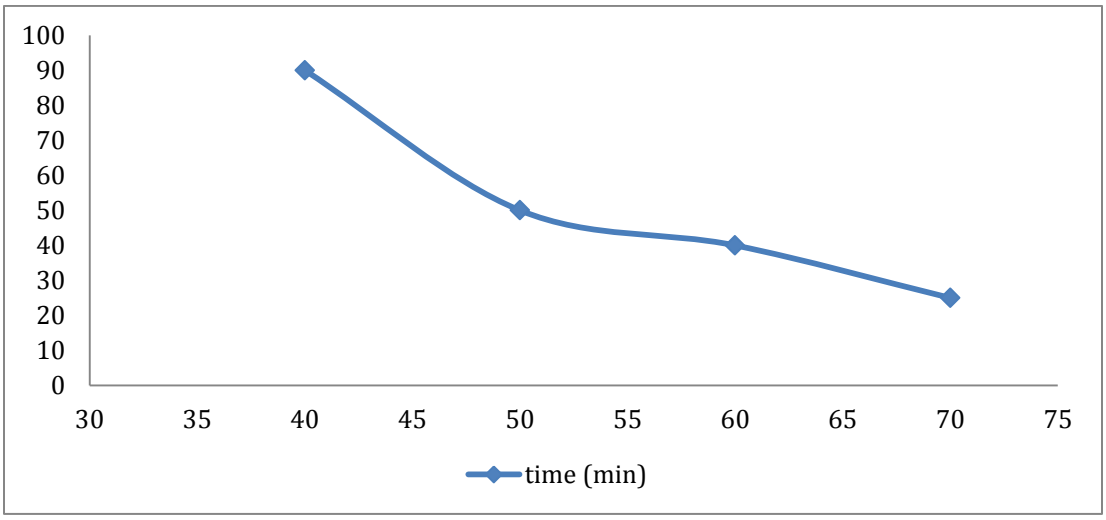


Figure 2. Effect of temperature on time needed for curcumin extraction.

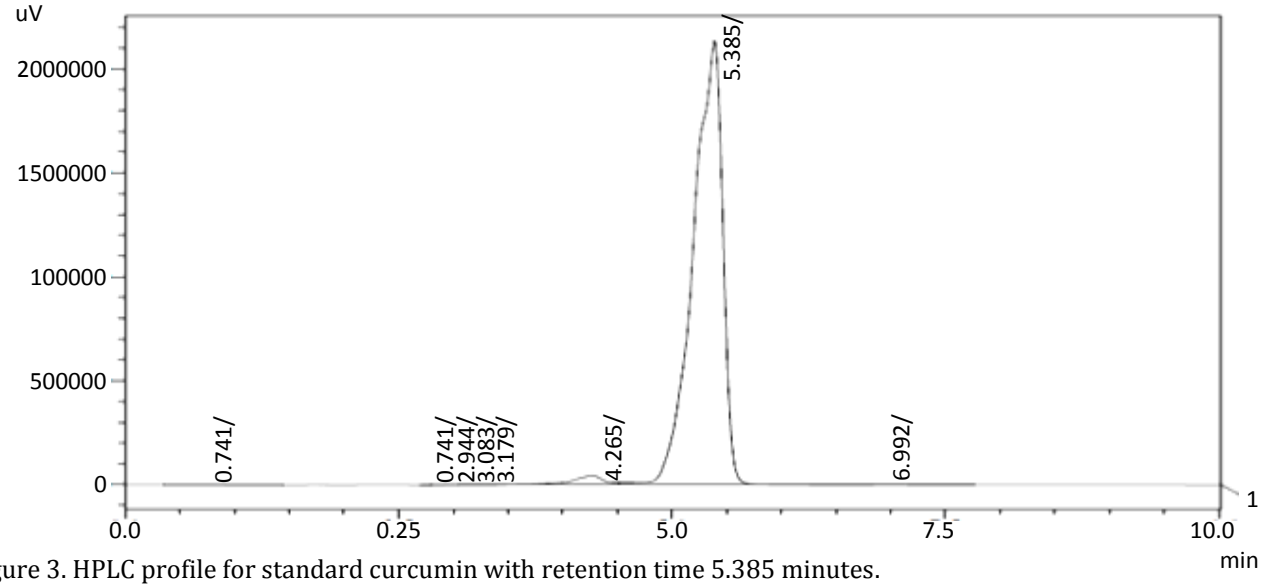


Figure 3. HPLC profile for standard curcumin with retention time 5.385 minutes.

Based from Figure 3, the highest peak showed the existence of curcumin at retention time 5.385 minutes in the standard with 98% purity procured from Sigma Aldrich (Albuch, Germany). Higher concentration of curcumin with higher injection volume produced larger peak area (Kamble *et al.,* 2011).

Curcumin sample analysis: The retention times obtained from HPLC analysis for all curcumin samples and curcumin standard were quite similar, which ranged from 5.385 to 5.862 (Figure 3 to Figure 7). The results showed similarities on the peak observed because the HPLC conditions used in this experiment were fixed for each samples. This showed that curcumin existed in all of the samples. In a study done by Revathy *et al.* (2011), curcumin was also found to be the major component in all the HPLC tested *Curcuma longa* samples.

According to Table 2 and Figure 8, HPLC analysis for each result showed slight difference in the percentage area of curcumin. When percentage area was high, the active compound which was curcumin in that sample

was also high. Sample in 50°C had the highest percentage area which was 92.304%, while sample in 60°C had the lowest which was 90.428%. But each of the samples did show high content of curcumin since *Curcuma Longa* does have higher curcumin content. On the other side, HPLC analysis for each result showed huge differences in the peak area of curcumin. Higher peak area showed a higher curcumin content. The 50°C sample showed the highest peak area with 10865498 mAU while the lowest peak area was the 40°C samples with 140107 mAU.

Table 2. Peak area of curcumin and percentage area of curcumin.

Sample	% Area of Curcumin	Peak area of Curcumin (Mau)
40°C	90.477	140107
50°C	92.304	10865498
60°C	90.428	3576304
70°C	92.053	414497

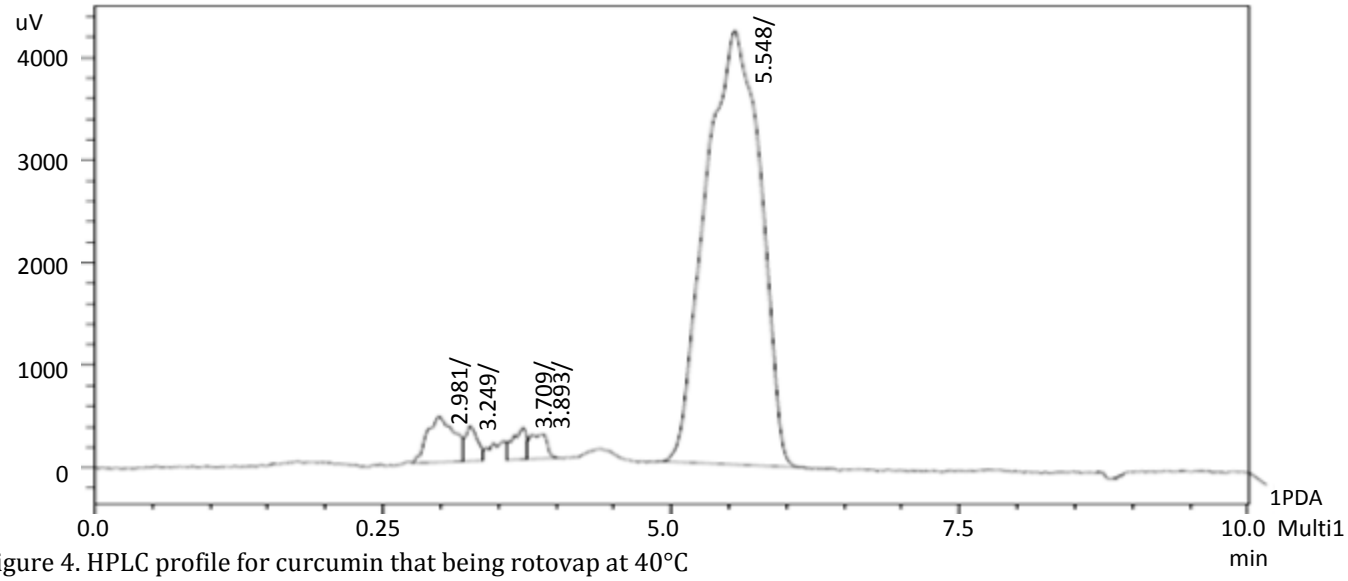


Figure 4. HPLC profile for curcumin that being rotovap at 40°C

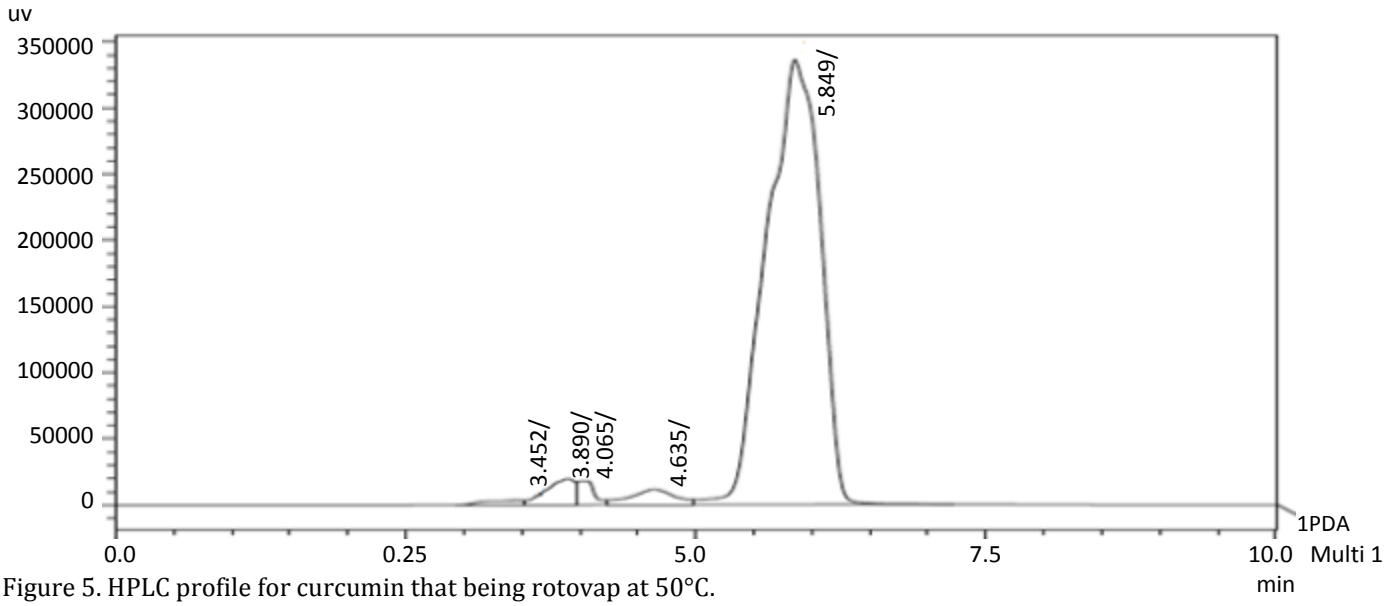


Figure 5. HPLC profile for curcumin that being rotovap at 50°C.

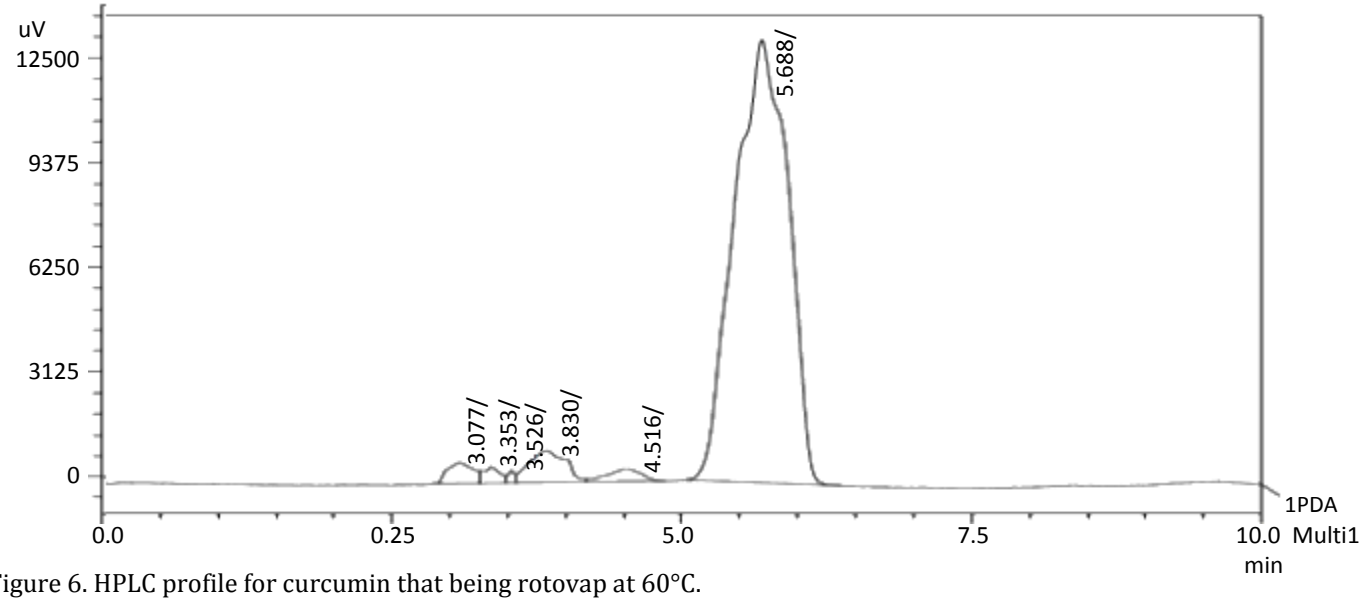


Figure 6. HPLC profile for curcumin that being rotovap at 60°C.

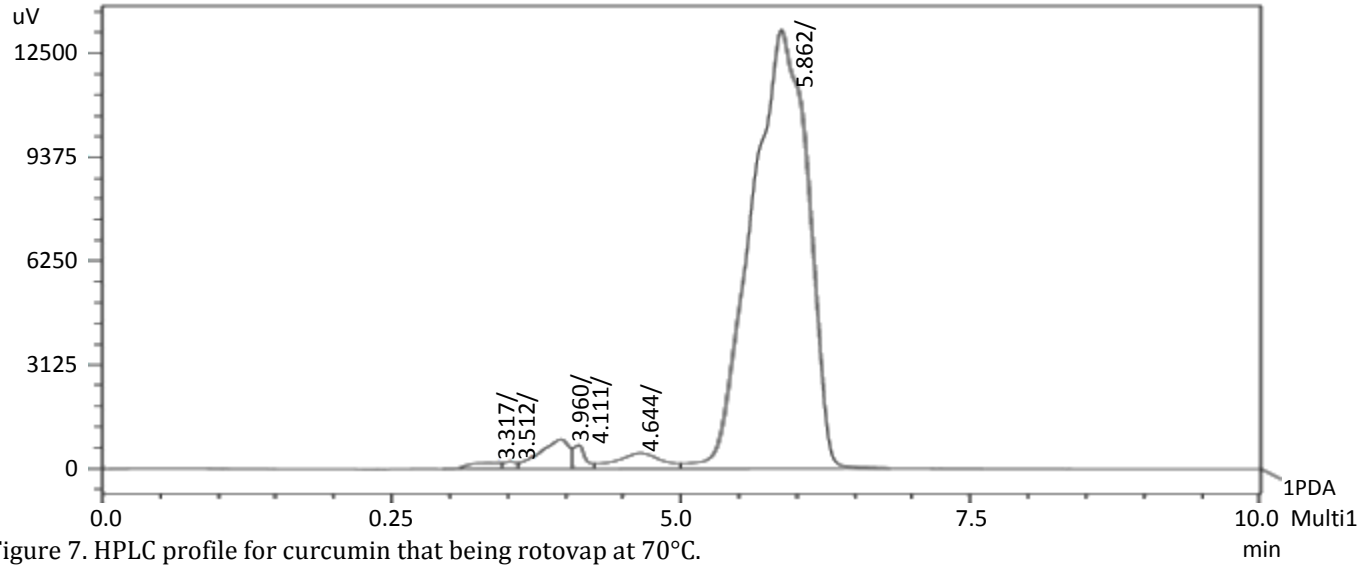


Figure 7. HPLC profile for curcumin that being rotovap at 70°C.

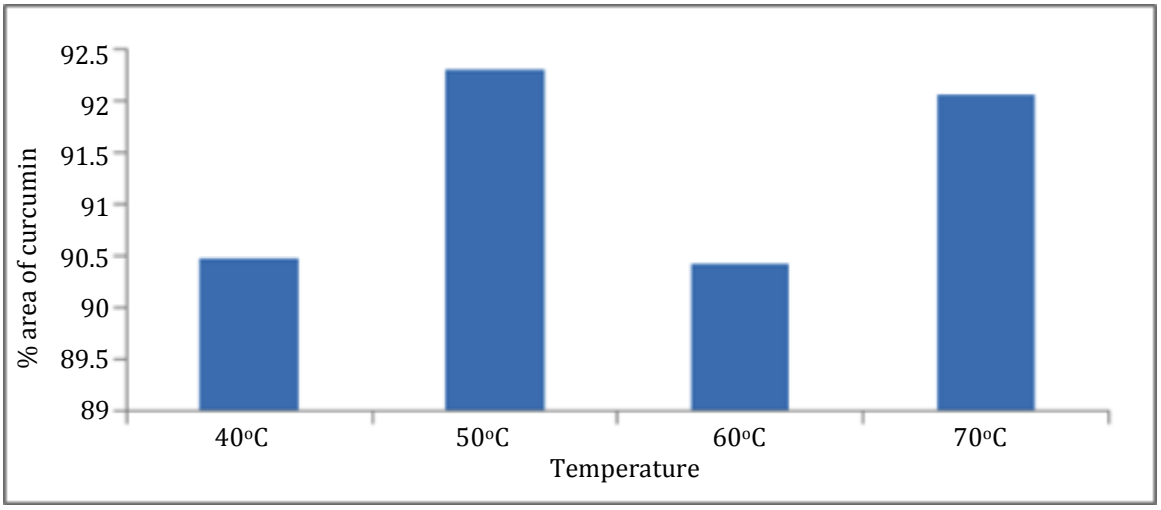


Figure 8. Percentage area of curcumin

UV Spectrophotometer analysis: In the study, the antioxidant activities of curcumin in various temperatures were evaluated and analyzed using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay method. DPPH method is usually used to test the ability of compounds to act as free radical scavengers or hydrogen donor and at the same time to evaluate antioxidant activity of foods. A measure of total antioxidants capacity helps to understand the functional properties of foods.

The antiradical activity was expressed as IC_{50} (µg/mL), the antiradical dose required to cause 50% inhibition. A lower IC_{50} value corresponds to a higher antioxidant activity of plant extract. The samples' concentrations against percentage of radical scavenging activity with standard deviation (% inhibition) were shown in Table 3. The DPPH of radical scavenging activity for 40°C curcumin extract at concentrations 6.25, 12.5, 25.0, 50.0 and 100.0 µg/ml were 4.71 ± 0.021, 9.13 ± 0.011, 18.32 ± 0.015, 33.92 ± 0.016 and 61.13 ± 0.006 respectively as shown in Figure 9 while DPPH of radical scavenging activity for 50°C curcumin extract at concentrations 6.25, 12.5, 25.0, 50.0 and 100.0 µg/ml were 15.43 ± 0.01, 26.91 ± 0.01, 50.06 ± 0.02, 77.86 ± 0.01 and 87.51 ± 0.001 respectively as shown in Figure 10.

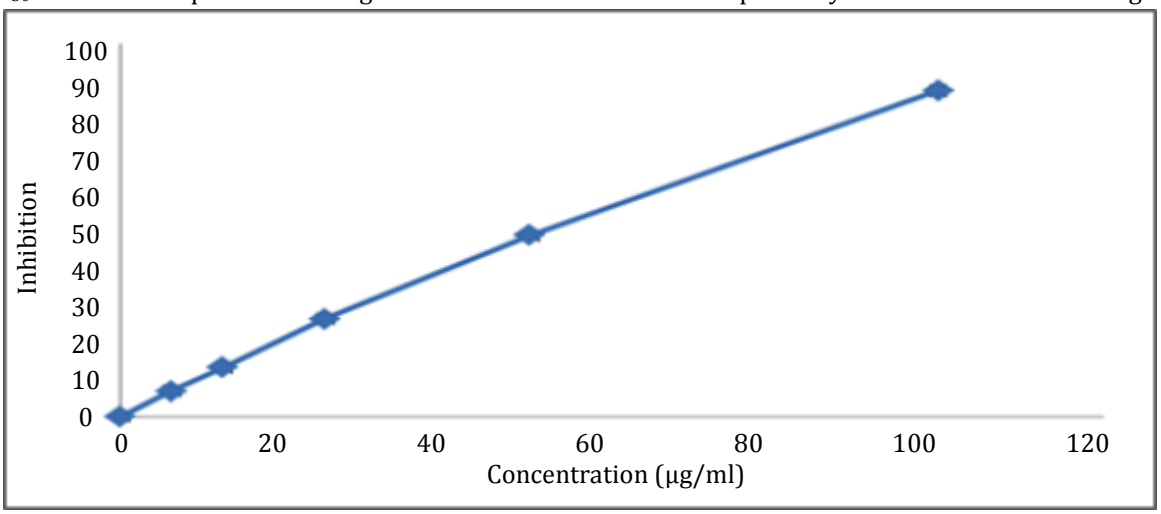


Figure 9. DPPH radical scavenging activity of 40°C curcumin extract.

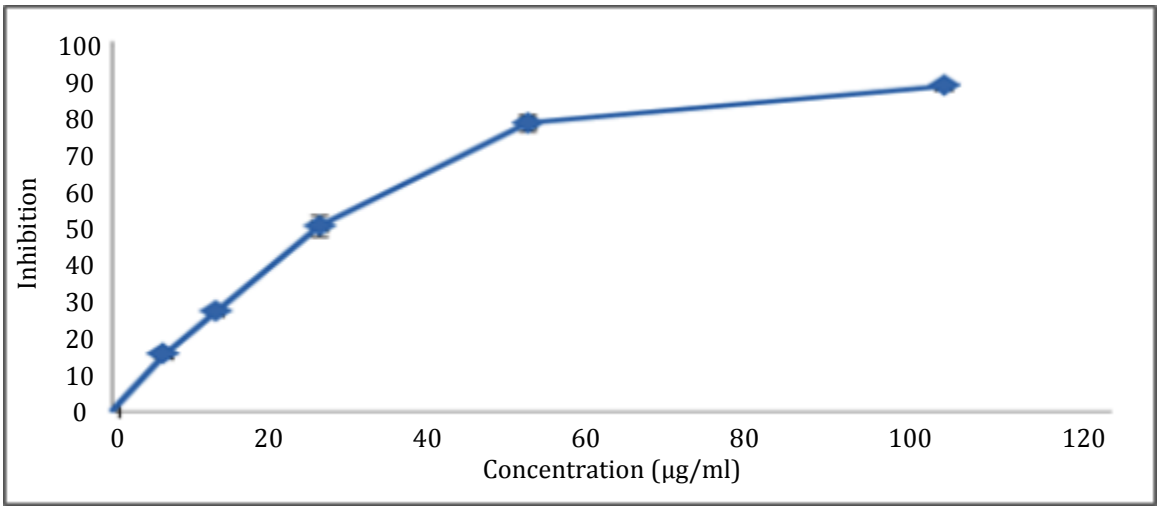


Figure 10. DPPH radical scavenging activity of 50°C curcumin extract

The DPPH of radical scavenging activity for 60°C curcumin extract at concentrations 6.25, 12.5, 25.0, 50.0 and 100.0 µg/ml were 7.66 ± 0.02, 14.78 ± 0.01, 27.74 ± 0.02, 49.29 ± 0.01 and 80.39 ± 0.003 respectively as shown in Figure 11 while DPPH of radical scavenging activity for 70°C curcumin extract at concentrations 6.25, 12.5, 25.0, 50.0 and 100.0 µg/ml were 0 ± 0.02, 3.59 ± 0.04, 8.83 ± 0.02, 19.73 ± 0.058 and 44.17 ± 0.04 respectively as shown in Figure 12.

From Figure 13, 70°C curcumin extract had the highest

value of IC_{50} which was 111.93±18.56 followed by 40°C curcumin extract which was 79.574±5.43, 60°C curcumin extract which was 51.137±4.56 and the lowest value of IC_{50} was the 50°C curcumin extract with 24.968±2.11 while IC_{50} for Ascorbid Acid was 5.202±0.38. 50°C curcumin extract had the highest antioxidant activity followed by 60°C curcumin extract, 40°C curcumin extract and 70°C curcumin extract. This showed that the 70°C curcumin extract had the lowest antioxidant activity.

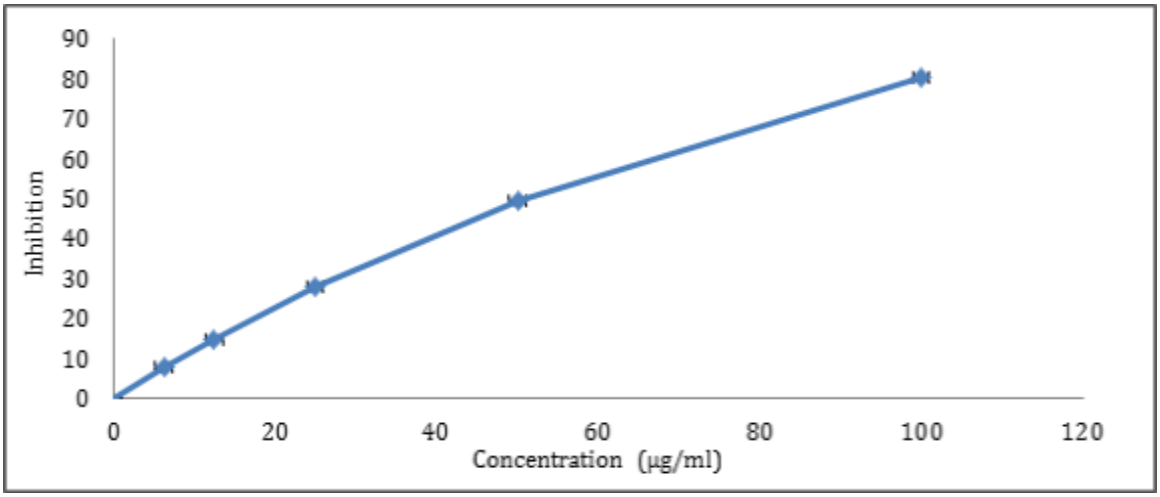


Figure 11. DPPH radical scavenging activity of 60°C curcumin extract.

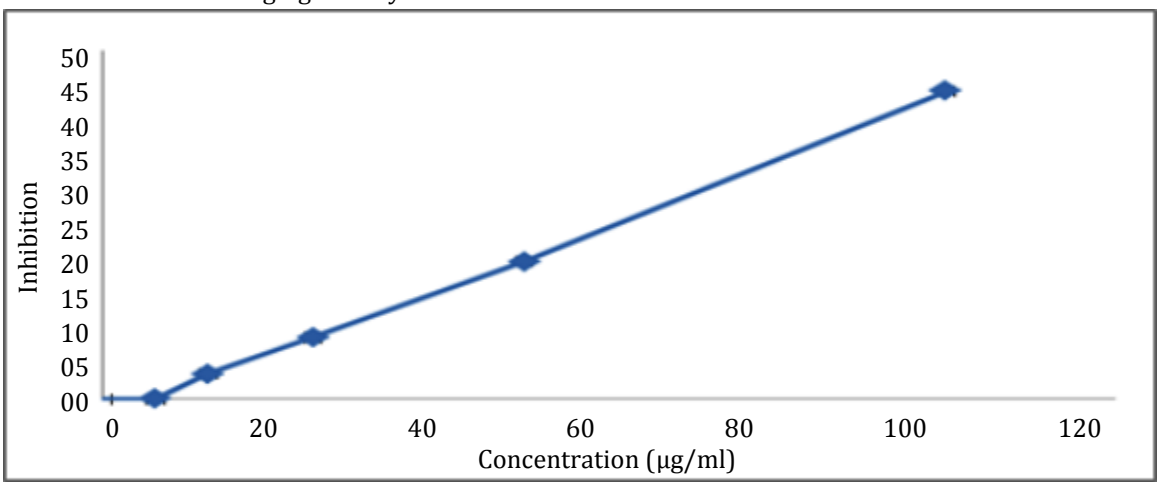


Figure 12. DPPH radical scavenging activity of 70°C curcumin extract.

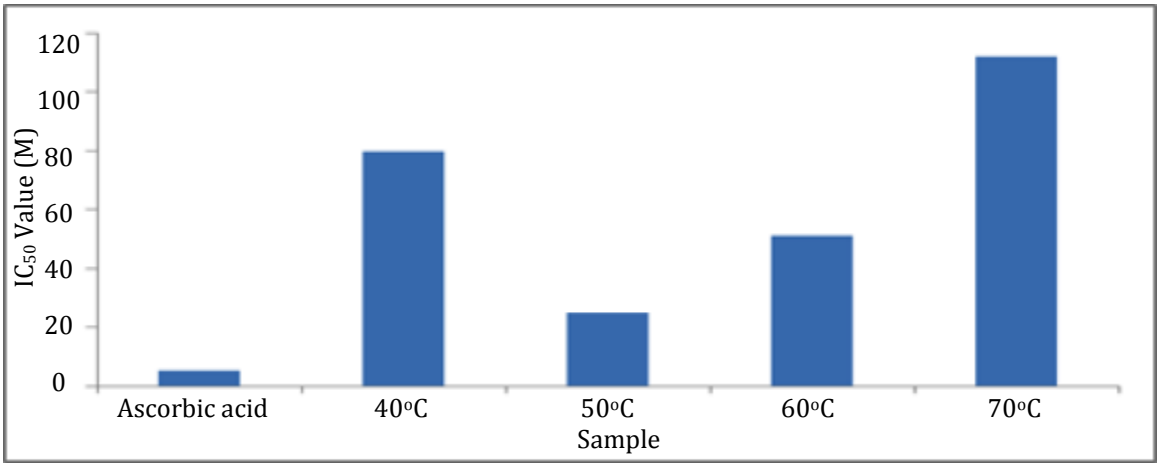


Figure 13. IC50 of Curcumin extracts and ascorbic acid.

Table 3. DPPH radical scavenging activity of Curcumin extracts at different temperature (°C).

Curcumin Extract (°C)	Concentration (µg/ml)	% Inhibition ± SD
40	0	0
	6.25	4.71 ± 0.02
	12.5	9.13 ± 0.01
	25	18.32 ± 0.02
	50	33.92 ± 0.02
	100	61.13 ± 0.01
50	0	0
	6.25	15.43 ± 0.01
	12.5	26.91 ± 0.01
	25	50.06 ± 0.02
	50	77.86 ± 0.01
	100	87.51 ± 0.001
60	0	0
	6.25	7.66 ± 0.02
	12.5	14.78 ± 0.01
	25	27.74 ± 0.02
	50	49.29 ± 0.01
	100	80.39 ± 0.003
70	0	0
	6.25	0 ± 0.02
	12.5	3.59 ± 0.04
	25	8.83 ± 0.02
	50	19.73 ± 0.058
	100	44.17 ± 0.04

The presence of phytochemical compounds such as alkaloid, flavonoid, saponin, tannin and terpenoid compounds might contribute to antioxidant activities. Besides, several studies reported that the phenolic compounds in spices and herbs significantly contribute to their antioxidant properties.

Statistical analysis: T-test analysis was used to compare the IC_{50} of the samples with ascorbic acid (standard) because they have different final concentrations. The T-test results showed that, P-value of Ascorbic Acid with 40°C, 50°C, 60°C and 70°C curcumin extract were 0.0009, 0.0016, 0.0014 and 0.0044 respectively, ($p \leq 0.01$) which was significantly different in IC_{50}.

T-test analysis also used to compare the mean inhibition of radical scavenging activity between the samples. The P-value of 40°C curcumin extract and 50°C curcumin extract was 0.00067 ($p \leq 0.01$) which was significantly different in inhibition of radical scavenging activity while P-value of 40°C and 60°C was 0.013 ($p \geq 0.01$) which was not significantly different in inhibition of radical scavenging activity.

The P-value of 40°C curcumin extract and 70°C curcumin extract was 0.018 ($p \geq 0.01$) which was not significantly different in inhibition of radical scavenging activity while P-value of 50°C curcumin extract and 60°C curcumin extract was 0.006 ($p \leq 0.01$) which was significantly different in inhibition of radical scavenging activity. The P-value of 50°C curcumin extract and 70°C curcumin extract was 0.005 ($p \leq 0.01$) which was significantly different in inhibition of radical scavenging activity while P-value of 60°C curcumin extract and 70°C curcumin extract was 0.015 ($p \geq 0.01$) which meant it was not significantly different in inhibition of radical scavenging activity.

From the experiment, most of the P-values for curcumin extract were significantly different which meant that there were slight differences in inhibition of radical scavenging activity among the samples. This also showed that the antioxidant activities do exist in all of the curcumin samples.

CONCLUSION

Solvent extraction is a more suitable method to extract the essential oil of *Curcuma Longa* compared to steam distillation and soxhlet extraction because solvent extraction does not require heat. Too much heat could damage antioxidant activity since antioxidant activity is necessary to analyze in this experiment. The retention times of the samples were slightly similar with the curcumin standard. From the experiment conducted, the sample extracts showed that the *Curcuma Longa* contained high amount of curcumin in all samples (40°C, 50°C, 60°C and 70°C curcumin extracts). Curcumin extract at 40°C showed 90.477% area of peak, 50°C curcumin extract with 92.304%, 60°C curcumin extract with 90.428% and 70°C curcumin extract showed 92.053% area of peak. The percentages indicated the curcumin extracts in the samples.

The 50°C curcumin extract showed the highest antioxidant activity with 24.968 IC_{50} value while the lowest was 70°C curcumin extract with 111.93 IC_{50} value. Curcumin and its derivatives can be further analyzed to determine their activities such as antioxidant and antibacterial properties. There are many modern methods such as Solid Phase Extraction (SPE) which also carries out the same function as an alternative to the conventional method of essential oils

purification such as Soxhlet extraction. This modern method provides many advantages which are easier, save cost and time and more efficient than the conventional method that requires a lot of solvent and is very time consuming.

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