Malays. Appl. Biol. (2019) 48(1): 123–129

A PRELIMINARY STUDY OF PHYTOCHEMICAL CONTENTS IN COMMERCIAL MALAYSIAN BLACK TEA EXTRACT USING QUANTITATIVE AND QUALITATIVE TESTS

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Accepted 9 January 2019, Published online 20 March 2019

ABSTRACT

Black tea (*Camellia sinensis*) is a flavoured, functional, and therapeutic non-alcoholic drink that is consumed by two-thirds of the world's population. In this study, the bioactive compounds in black tea leaves were extracted using two different solvents which are methanol and acetone before being further characterized with FTIR to identify the functional groups. The presence of active compounds such as gallic tannin, catecholin tannin, phenol and flavonoid compounds were further tested with qualitative and quantitative methods. Based on FTIR results, the best solvent to extract tea leaves was acetone. The frequency obtained for phenolic –OH stretch was 3390.5 cm^{-1}, while alkane $–CH_3$ stretch appeared at 2921.4 cm^{-1} and 2850.7 cm^{-1}. The UV-Vis spectrum was obtained and the λ max from the spectrum is 270 nm. Qualitative analysis involving ferric chloride and lead acetate test showed positive results for the presence of gallic tannin, catecholin, flavonoid and phenols in the tea leaves extract. From the quantitative test, the total phenolic content was 0.9217 mg gallic acid equivalent (GAE)/L while the concentration of flavonoid content was 0.7792 mg quercetin/L and total tannin content 0.13 g/g respectively.

Key words: Organic extracts, black tea, phytochemical, phenolic, flavonoid

INTRODUCTION

Tea from the leaves of *Camellia sinensis*, a plant from the *Theaceae* family which has been cultivated in more than 30 countries, is known as the most popular beverage in the world (Loto, 2011). According to World Tea News (2013), Tea originated from China in 2737 BC and tea plantation was introduced to Malaysia in the year 1929. In Malaysia, Malays prefer strong tea (80% of consumption is black tea), while Chinese consumers tend to prefer light Chinese teas such as tie kuan yin, puer, oolong and green tea (World Tea News, 2013). Global tea consumption can mainly be divided into three types of tea which are black tea, green tea, and Oolong tea, 78%, 20%, and 2% respectively (Sharangi *et al.,* 2014). Black tea is made from fully fermented leaves while green tea is produced by steaming or panning methods to prevent oxidation and retain the green colour. The price range of tea in Malaysia is from RM9.73 to RM12.39 per kilogram (Indexmundi.com, 2018).

The presence of bioactive phytochemical constituents in *C. sinensis* present many benefits to humans such as anti-inflammatory activities and is a strong antioxidant (Bhutia *et al.,* 2015). Some examples of beneficial phytochemical constituents includes flavonoid, phenolic and tannin. These chemical compounds possess the ability to neutralize free radicals in the body by sequestering metal ions and by scavenging reactive oxygen and nitrogen species (Pisoschi & Negulescu, 2011). However, the content of phytochemicals can vary with tea fermentation method used, climate, horticultural practices, leaf age and leaf variety. Thus, the purpose of this study is to extract and characterize commercial Malaysian black tea leaves (BOH brand) by using FTIR and UV-Vis and to identify the phytochemical compounds contained in commercial black tea using qualitative (phytochemical screening test) and quantitative method (calibration curve method).

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MATERIALS AND METHODS

Preparation of tea leaves extract

For extraction preparation, 500 of powder was obtained from commercially tea leaves (BOH brand). It was first soaked for 7 days in 500 mL methanol in 1 L glass flat bottom flask. Then, the mixture was filtered through a Whatman filter paper cat no 1001-125 Qualitative Filter Paper Circles, 11 Micron. The filtrate was then temporarily kept in 1 L blue cap bottle. The steps from soaking until filtration were repeated for another 500 g of tea powder. Both filtrates were collected and subjected to rotary evaporation to remove the methanol.

During the evaporation process, the temperature was set at 60°C and was centrifuged at 100 rpm until all the solvent evaporated. After the crude extract was obtained, the extract was kept enclosed at room temperature in 20 mL borosilicate glass scintillation vial until further process. In order to prevent potential safety hazards in the laboratory, all work involving hazardous or volatile materials was done under the fume hood. The extraction method was repeated with the different solvents.

Fourier Transform Infrared Spectroscopy (FTIR) test

The FTIR analysis technique was carried out using FTIR Spectrometer PerkinElmer. A small drop of crude sample was placed into the plate and put the plate into the sample holder. The spectrometer scans the sample within the frequency range of 4000 cm^{-1} to 400 cm^{-1} (Qian *et al.,* 2013). By observing the vibrational motion of bonds from the spectrum obtained and analyzing the spectral pattern according to IR absorption table, functional group contained in tea can be identified.

Ultraviolet Visible (UV-Vis) and calibration curve

The UV-Vis test was used to analyze the absorption and the λ-max for the compound. The model UV-1800 Shimadzu spectrophotometer was used to perform this test. The absorbance at transmission mode (200-800 nm) was monitored and the background solvent used was acetone. For the sample preparation, the crude sample was diluted with acetone before the sample was put into the cuvette to be analysed by the UV-Vis spectrometer.

Qualitative test of black tea leaves extract

Phytochemical screening test

After extraction, phytochemical screening tests were conducted. This test was conducted to detect the presence of bioactive compounds in black tea including tannin, phenolics and flavonoids. Ferric chloride test was conducted to detect catecholic tannin, gallic tannin and phenolic compound while lead acetate test was conducted to detect flavonoid compound. To determine the presence of either catecholic or gallic tannin, a small quantity of 0.5 mL of extract was mixed with 2 drops of ferric chloride to be used. A change in the colour of the mixture was then observed where a blue colour indicated the presence of gallic tannin and a green-black colour indicated the presence of catecholic tannin. While 1 mL of distilled water and 2 mL of ferric with 0.5 mL extract detected the presence of phenolic compound. The blue, red or purple colour showed the presence of phenolic. White colour represented the flavonoid content when 2 mL of extract was treated with 3 drops of lead acetate (Tariq & Riyaz, 2012).

Quantitative tests of black tea leaves extract

To measure the amount of phenolic and flavanoid contents in the tea extract, the calibration curve method was used. In this method, gallic acid and quercetin had been used. The total phenolic content (TPC) and total flavonoid content (TFC) was calculated and the standard curve equation was $y = mx+c$.

For quantitative tests of the tea leaves extract, phenolic, flavonoid and tannin contents were measured.

Total phenolic content (TPC)

The total phenolic content was determined using the Folin–Ciocalteu method in term of gallic acid equivalent (GAE) in mg/L of extract (Mohd Zain & Wan Omar, 2018, Liebert *et al.,* 1999). The gallic acid stock solution was first prepared with 0.125 g gallic acid and dissolved within 10 mL of methanol in 25 mL volumetric flask. Then a series of working solutions were prepared with 6 different concentrations which are 0, 10, 20, 30, 40, 50 mg/L. These working solutions were used to construct a standard curve where the absorbance for each solution was determined at 760 nm using a UV-Vis spectrometer. For the determination of the TPC of the tea leaves extract, 9 mL of distilled water, and 1 mL of 10% Folin-Ciocalteu‘s reagent were mixed and the mixture was then shaken well. After 5 minutes, the mixture was added with 10 mL of 7.5% $NaHCO_3$ and distilled water was added to the mark. After that, the sample was incubated for 90 minutes at room temperature and the absorbance of the mixture was measured 750 nm. The content of gallic acid in the extract was then compared from the standard curve of gallic acid constructed above.

Total flavonoid content (TFC)

First, 0.125 g quercetin was dissolved in 10 mL methanol to make a quercetin stock solution. A series of working solutions were then prepared from this stock solution (i.e. 0, 10, 20, 30, 40 and 50

mg/L). These working solutions were used to construct a quercetin standard curve. The absorbance of each solution was determined at 415 nm using a UV-Vis spectrophotometer. To determine the TFC of tea leaves extract, 1 mL of calibration solution was dissolved with 4 mL of distilled water, 0.3 mL of 5% $NaNO_2$. After 5 minutes, 0.3 mL of 2% $AlCl_3$ solution was dissolved in the volumetric flask before analysing with UV-Vis. The samples were incubated for an hour at room temperature. The absorbance of quercetin was determined by using spectrophotometer at the wavelength obtained from UV-Vis. The samples were prepared in triplicates. The standard solution of quercetin was prepared and the calibration line was constructed by repeating the same procedure. The calibration line for the concentration of flavonoids based on the measured absorbance was read as mg/mL. Then, the content of flavonoids in extracts was expressed in terms of quercetin equivalent (mg of QE/L of extract) (Kalita *et al.,* 2013).

Total tannin content

The tannic acid was produced from the tea leaves in order to obtain specific tannin content. Based on the number of final and initial mass, the percentage of tannin was calculated from the tannic acid obtained. In this test, 10 g of dried BOH black tea was weighted in the beaker before 100 ml of deionized water was added. The tea leaves was boiled at 100°C for 20 min. The boiled solution was filtered using a filter funnel and filter paper with diameter 110 mm Filters Fioroni. In the filtrate, 2 g of calcium carbonate ($CaCO_3$) was added and the mixture was boiled until calcium tannate became visible. The precipitate solution was filtered again to obtain calcium tannate and residue of calcium tannate was hydrolysed with 5 mL concentrated HCl (0.25 M). The crystals of tannic acid were filtered, dried and weighted (Bizuayehu *et al.,* 2016).

RESULTS AND DISCUSSION

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) analysis method uses infrared light to scan test samples and observe chemical properties. Figure 1 shows the FTIR spectrum of tea leaves concentration that consisted of 5 mL tea extract. From the IR spectra, it is shown that these tea leaves extract consisted of phenols C_6H_5OH, hydroxyl O-H, alkene C=C, alkane C-H and amide C=O functional groups (Figure 2). Indeed, this was the main component that can be found in the tannin structure. The examples of the tannin compounds are theaflavin, theaflavin monogallate and theaflavin digallate as shown in Figure 3. From the FTIR results, it's also shows that the functional groups for TPC and TFC consisting of aromatic rings and hydroxyl group possess strong antioxidant properties (Table 1 & Table 2). The broad absorbance in the Figure 1 at range 3000 cm^{-1} -3300 cm^{-1} is due to the intermolecular hydrogen bonding between the OH group and adjacent oxygen atom (Uddin *et al.,* 2011). The scavenging potential of phenolic substances might be due to the active hydrogen donating ability of the hydroxyl substitution (Sentilkumar *et al.,* 2017). This was further supported by preliminary studies on the correlation of antioxidant activity versus TPC and TFC (Velioglu *et al.,* 1998, Sangeetha & Vijayalakshmi, 2011).

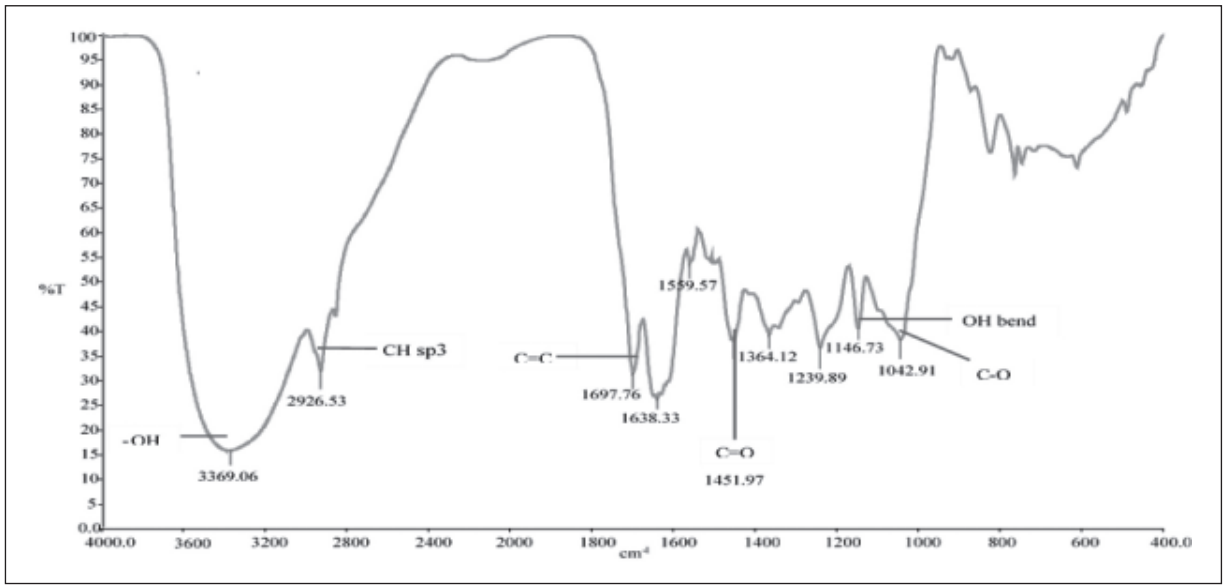

Fig. 1. IR spectrum of tea extracted with acetone.

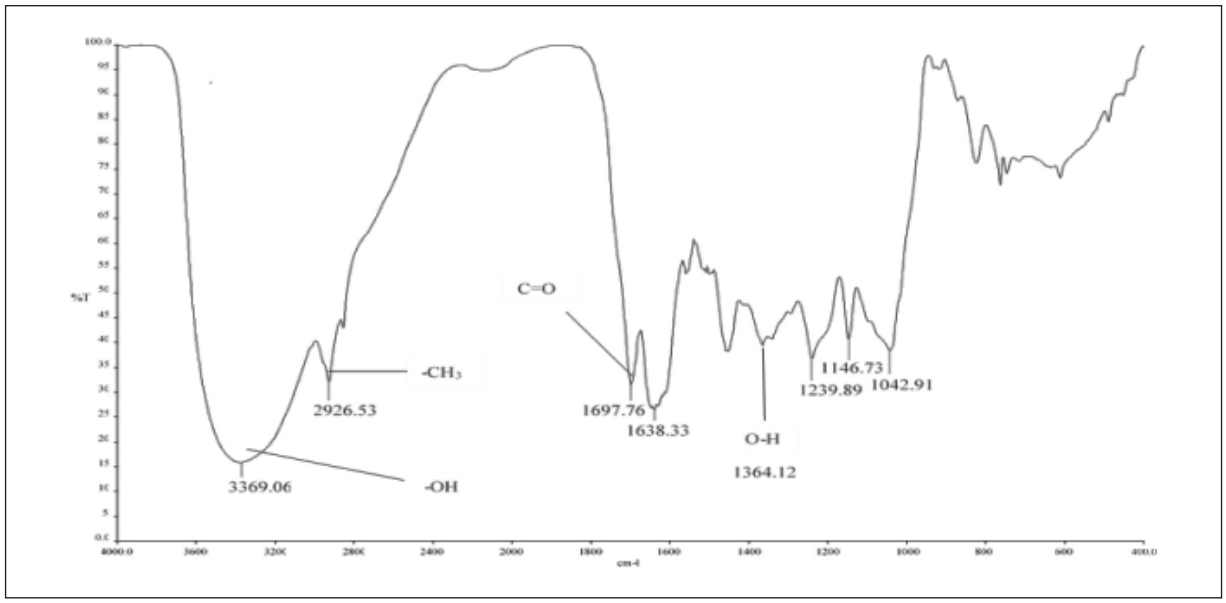

Fig. 2. IR spectrum of tea extracted with methanol.

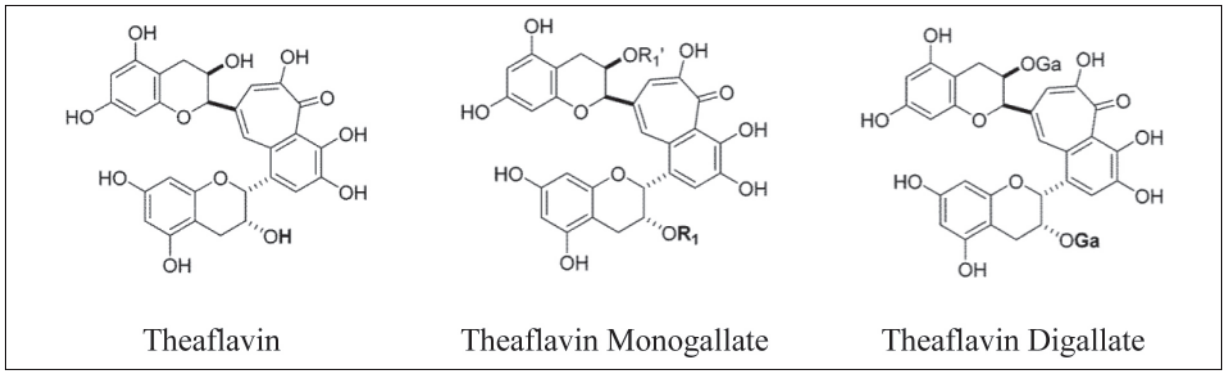

Fig. 3. Different compound of tannin.

Table 1. FTIR of tea extracted with acetone

No.	Abs (cm^{-1})	Functional Group	Intensity
1.	3390.54	-OH	Broad
2.	2926.39	$-CH_3$	Strong
3.	2850.72	$-CH_3$	Strong
4.	1695.9	C=O	Strong
5.	1644.9	C=O	Medium
6.	1364.86	-OH	Medium
7.	1239.5	C-N	Medium
8.	1147.9	C-O	Strong

Table 2. IR spectrum of tea extracted with methanol

No.	Absorbance	Functional Group	Intensity
1.	3369.1	-OH	Strong and broad
2.	2926.5	$-CH_3$	Medium
3.	1697.8	C=O	Strong
4.	1364.1	O-H	Medium

Ultraviolet Visible (UV-Vis)

The UV-Vis test results indicated the absorption and the λ-max (270 nm) (Table 3) of the compound in the tea leaves (Figure 4). This wavelength was further used in the determination of the compound for the quantitative test (calibration curve). The absorbance was calculated from the wavelength.

Qualitative phytochemical test

Phytochemical screening test

The easiest way to determine the presence of chemical compounds is by conducting the

Table 3. Data from UV-Vis spectra

No.	Wavelength nm	Absorbance
1.	660.50	0.005
2.	270.00	0.072
3.	209.00	0.557

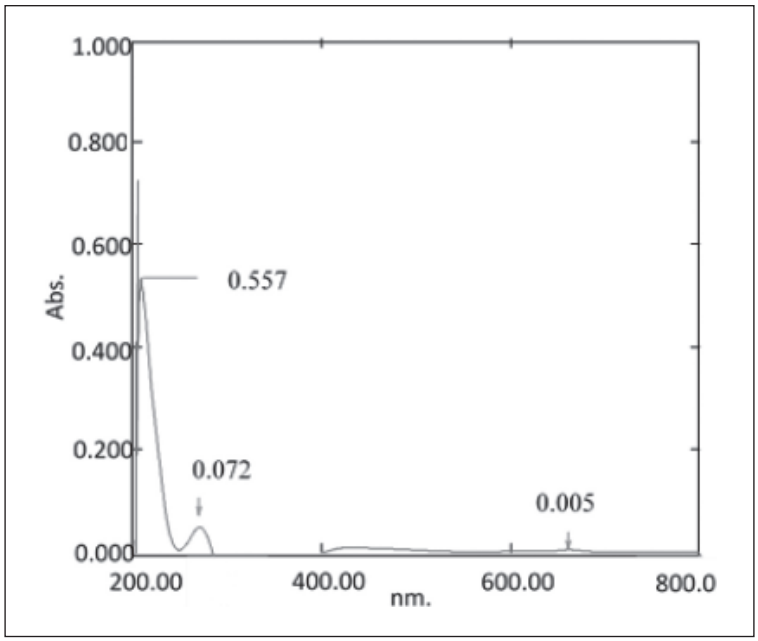

Fig. 4. UV-Vis spectrum for tea.

Table 4. Table of compound test and colour changes

Compound	Chemicaltest	Colour Change	
		Initial	Final
Gallic Tannin	Ferric Chloride	Dark	Blue
Phenols	Ferric Chloride	Dark	Blue
Catecholin	Ferric Chloride	Dark	Green black
Flavonoid	Lead Acetate Solution	Dark	Colourless/white

phytochemical test. This includes ferric chloride and lead acetate test.

The results in Table 4 specified the presence of tannin, phenols, catecholin and flavonoid compounds. Ferric chloride test was conducted to detect the presence of gallic tannin, catecholin tannin and phenols. The results of gallic tannin and catecholin tannin were positive where the colour changed to blue and green black respectively. While for phenols show positive results when the reaction turned to blue colour. Lastly, lead acetate test gave positive results by forming white colour solution. This shows significant presence of flavonoids.

Quantitative phytochemical test

Tannin content

From the qualitative test, it showed the presence of tannin content in the black tea extract. The quantitative test was to find the exact amount of the compound in the black tea leaves. The test used 10.0 g of tea leaves powder as initial weight. As a result, 0.13 g/g of tannic acid was obtained (Table 5).

Table 5. Percentage of tannic acid obtained

Type of Tea	Black tea
Initial weight of tea leaves (g)	10.0
Final weight Tannic acid (g	1.30
Percentage of tannin obtained (%)	13.3

Phenolic and flavonoid content

The data from UV-Vis spectra was used to calculate TPC and TFC in tea leaves extract. The absorbance of phenolic and flavonoid in concentration mg/L is shown in Figure 5 and 6. The TPC was 0.9217 mg (GAE)/L while the concentration of TFC was 0.7792 mg (QU)/L. From the quantitative test, the result proved that the phenolics and flavonoids are present in local black tea. To compare with the previous studies, the TFC in black tea was 6.78 mg GAE/L (Nibir *et al.,* 2017). While for the concentration of flavonoid was 2.19 mg (QU)/L (Dwyer *et al.,* 2018). The phenolics and flavonoids are also present in the previous study but the values

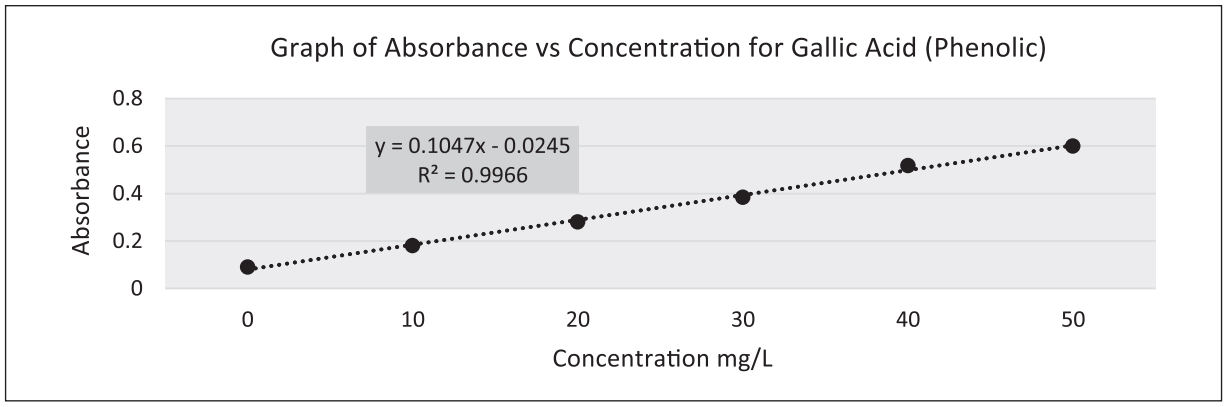

Fig. 5. Graph of absorbance vs concentration for Gallic Acid.

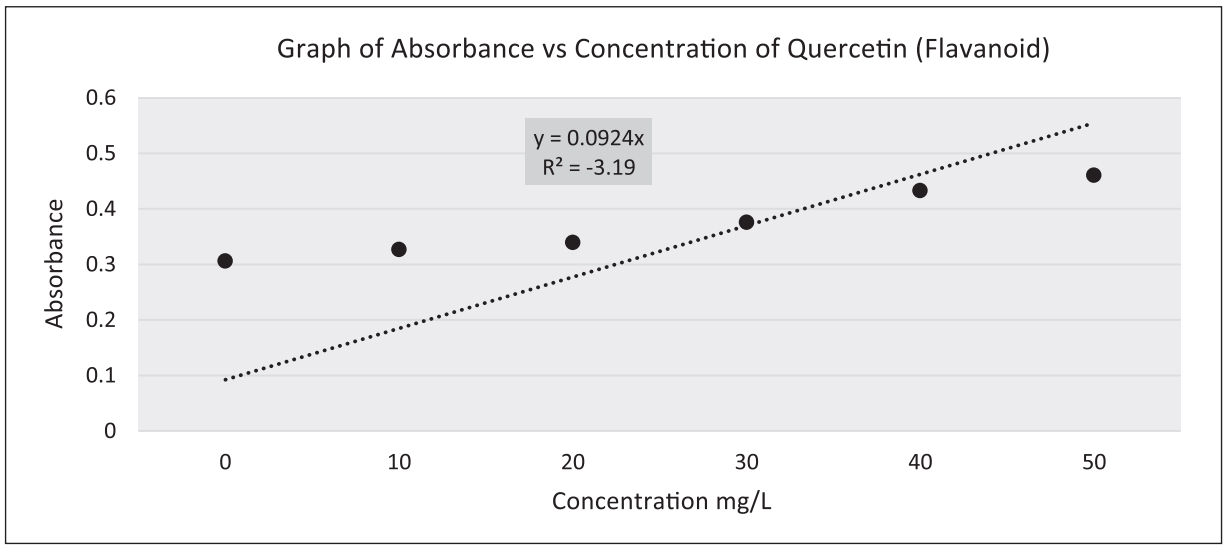

Fig. 6. Graph of absorbance versus concentration for Quercetin.

are much higher. This is because the origin of black tea used is from different countries that have the different climate that may affect the concentrations obtained.

As conclusion, the FTIR results shows acetone is the best solvent to extract tea leaves which gives the best frequency of functional group after being analysed and was supported by phytochemical screening test. The frequency obtained indicated the presence of gallic tannin, catecholin tannin, phenol and flavonoid. From the quantitative tests, the TFC was 0.9217 mg (GAE)/L while the TFC was 0.7792 mg QU/L and total tannin content of 0.13 g/g respectively. This shows that the highest concentration of bioactive compounds in crude black tea extract was tannin followed by phenolics and flavonoids. For future work, the tea leaves extracted solution need to be further purifed with column chromatography and then analysed with NMR and HPLC to get more accurate results.

ACKNOWLEDGEMENT

The author would like to thank the School of Fundamental Science, University Malaysia Terengganu (UMT) for their assistance and support in conducting this work.

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