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Antioxidant Activity, Phenolic, Flavonoid and Tannin Content of Piper Betle and Leucosyke Capitella

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ABSTRACT

Piper betle and *Leucosyke capitella* are plants that are commonly used as traditional medicine. In this study, antioxidant Activity, Phenolic, Flavonoids and Tannin content of these plants were evaluated. The plants were extracted using petroleum ether, acetone and methanol. The total phenolic, flavonoid and tannin extract of both plants is in the order of acetone > methanol > petroleum ether. The result shows that the total phenolic, flavonoid content for *Piper Betle* and *Leucosyke Capitella* is in the range of 31.25 to 47.48 mg/g and 1.68 to 7.19 mg/g, respectively. For *Piper betle*, the total flavonoid content is in the range of 29.58 to 46.08 mg/g and for *Leucosyke capitella* is in the range of 1.08 to 6.83 mg/g. It is also found that *Piper betle* extracted with methanol has higher antioxidant activity than vitamin E, butylated hydroxytoluene (BHT) and catechin but lower than quercetin.

| antioxidant Activity | Phenolic | Flavonoids | *Leucosyke Capitella* | *Piper Betle* |

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1. INTRODUCTION

Antioxidant compounds play an important role in limiting the damaging effect of free radicals and retard the process of many chronic diseases as well as lipid oxidative rancidity in foods [1]. Plants exhibit different antioxidant capacities according to their polyphenol content, vitamin C, vitamin E, carotenoids and flavonoids [2]. Phenolics compounds are a large diverse group of secondary plant metabolites including phenolic acids, flavonoids, and tannins[3]. Flavonoids are polyphenolic compounds that are the most abundant of the plant phenolics [3]. Tannins are phenolic compounds that shown potential antiviral, antibacterial and anticancer effects [4]. Many of these phytochemicals are found in plant that function with nutrients and dietary fiber and act as natural antioxidant.

Piper betle and *Leucosyke capitella* are plants that commonly used by indigenous people of Borneo for medicinal purpose. *Piper betle* also known as betel the leaf of a vine belonging to the *Piperaceae* family, while *Leucosyke capitella* is belonging to the *Urticaceae* family. In the traditional medicinal systems, the leaf of *Piper betle* has been advocated for the treatment of wounds, boils, bites of insects and for enhancing digestion [5]. Locally, betel leaves are used for chewing. The extract are also found to possess both superoxide and hydroxyl free radical scavenging action [5].

Leucosyke capitella or also known locally as *Mandahasi* grows at Borneo area (Sabah and Kalimantan, Indonesia). This plant is used by communities in Kalimantan to cure diarrhoea [6]. The leaf decoction is also used to treat hypertension and diabetes by Kadazandusun communities around Crocker Range [7].

Based on the traditional use of these plants in medicine, this research was conducted to determine its antioxidant activity, phenolic, flavonoid and tannin content. The relationship between antioxidant activity with phenolic, flavonoid and tannin content were also investigated.

2. EXPERIMENTAL

2.1 Chemicals and Reagents

Quercetin, catechin and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemical. Folin-Ciocalteu reagent was purchased from Sigma Aldrich. 4-hydroxy-3-methoxybenzaldehyde (Vanillin) was from Fluka Chemical. Aluminium chloride hexahydrate, $AlCl_3.6H_2O$ was obtained from Ajak Chemical while sodium carbonate, Na_2CO_3 from Merck. Acetone, methanol, hydrochloric acid, butylated hydroxytoluene (BHT) and vitamin E were purchased from QReC Chemical. Petroleum ether solvent was from Lab-Scan Analytical Science.

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2.2 Plant Materials

Piper betle was collected from Mentakab, Pahang while *Leucosyke capitella* was collected from Ranau, Sabah in November, 2008 and identified their scientific name by botanist Mr. Baharuddin Sulaiman, in the School of Biological Sciences at University Science of Malaysia. The whole both plants were dried for a few weeks under room temperature.

2.3 Plant Extracts

Dried leaves of *Piper betle* were ground into a fine powder with a grinder. Three solvents (petroleum ether, acetone and methanol) with distinct polarity were used for the preparation of plant extracts. 100 g of powdered leaves were extracted with 250 mL of petroleum ether in 500 mL conical flask for 2 days and this procedure was repeated with the same solvent once again. The aliquot were pooled and filtered. Then, the filtrate was evaporated down to dryness under vacumm using rotary evaporator at 40 °C. This petroleum ether extract was weighed and transferred to a freezer. This process was repeated with acetone and methanol using the plant residue obtained from petroleum ether extraction. The whole process was repeated for the next sample which is the dried stem of *Leucosyke capitella*.

2.4 Preparation of Stock Solution

0.05 g samples or standards were dissolved in 50 mL volumetric flask with solvent to prepare 1000 ppm of stock solution. The solvent used depends on the samples or the standards (Table 1).

Table 1 The solvent used for samples

Sample	***Solvent***
Piper betle petroleum ether crude	Acetone
Piper betle acetone crude	Acetone
Piper betle methanol crude	Methanol
Leucosyke capitella petroleum ether crude	Acetone
Leucosyke capitella acetone crude	Acetone
Leucosyke capitella methanol crude	Methanol

2.5 Total Phenolic Assay

Total phenolic content was determined using the modified Folin-Ciocalteu method. Eight concentrations (0, 5, 10, 20, 30, 40, 50 and 100 ppm) of catechin (standard) were prepared to obtained catechin standard calibration curve and 100 ppm of each sample was prepared to determine total phenolic content in catechin equivalent. 0.25 mL of samples or standards was diluted with 3.75 mL of distilled water followed by the addition of 0.25 mL of Folin-Ciocalteau reagent and left for 3 minutes. 1.25 mL of 20 % (w/v) sodium carbonate, Na_2CO_3 was added to the mixture, and then incubated for 40 minutes at 40 °C. The absorbance was measured at 685 nm using UV-Vis spectrometer.

2.6 Total Flavonoid Assay

Total flavonoid content was determined using the method of Quettier-Deleu. Eight concentrations (0, 5, 10, 20, 30, 40, 50 and 100 ppm) of quercetin (standard) were prepared to obtained quercetin standard calibration curve and 100 ppm of each sample was prepared to determine total flavonoid content in quercetin equivalent. 5 mL of samples or standards were mixed with 5 mL of 2 % (w/v) of aluminium chloride hexahydrate, $AlCl_3.6H_2O$. After 10 minutes, the absorbance was measured at 415 nm using UV-Vis spectrometer.

2.7 Total Tannin Assay

Total tannin content was determined using vanillin assay. Eight concentrations (0, 5, 10, 20, 30, 40, 50 and 100 ppm) of catechin (standard) were prepared to obtained catechin standard calibration curve and 100 ppm of each sample was prepared to determine total tannin content in catechin equivalent. 1.0 mL of samples or standards was mixed with 3.0 mL of 4 % (w/v) vanillin followed by addition of 1.5 mL of 1 M hydrochloric acid, HCl, then incubated in the dark for 5 minutes and the absorbance was measured at 500 nm using UV-Vis spectrometer.

2.8 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

This simple method was adapted to measure the radical scavenging activity of the extracted sample using the stable free radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH•). Eight concentrations (0, 5, 10, 20, 30, 40, 50 and 100 ppm) of catechin, quercetin, butylated hydroxytoluene (BHT), vitamin E and samples were prepared. 4.0 mL of samples or standards were mixed with 0.5 mL of 1 mM of DPPH. After 10 minutes, the absorbance was measured at 517 nm using UV-Vis spectrometer.

3. RESULTS & DISCUSSION

3.1 Total Phenolic Content

The Follin-Ciocalteu's assay is one of the oldest methods designed to determine the total content of phenolics in food or medicinal plantsm [8]. Phenolic compound react with Follin-Ciocalteu'Reagent (FCR) only under basic conditions. Dissociation of a phenolic proton in basic medium leads to a phenolate anion, which is capable of reducing FCR in which the molybdate in testing system is reduced forming a blue coloured molybdenum oxide with maximum absorption near 685 nm. The intensity of blue calouration produced is proportional to the total to the total quantity of phenolic compounds present in the testing samples [8,9].

The total phenolic content (TPC) of *Piper betle* ranged from 31.25 to 47.48 mg/g (Table 2) was determined at 100 ppm from standard calibration curve of catechin (Figure 1). The *Piper betle* extracted using acetone had the highest total phenolic content of 47.48 mg/g, followed by methanol of 34.29 mg/g and petroleum ether of 31.25 mg/g.

The total phenolic content of *Leucosyke capitella* ranged from 1.68 to 7.19 mg/g (Table 2). The *Leucosyke capitella* extracted using acetone had the highest total phenolic content of 7.19 mg/g, followed by methanol of 2.26 mg/g and petroleum ether 1.68 mg/g.

From the results, it shows that acetone extract more phenolic compound compares to methanol and petroleum ether. Besides, it also shows that acetone is more effective than methanol to extract polar phenolic compound for *Piper betle* and *Leucosyke capitella*. It is also noted that *Piper betle* contain more phenolic compound than *Leucosyke capitella.*

Table 2 Total phenolic, flavonoid and tannin contents in *Piper betle* and *Leucosyke capitella*

Sample	***TPC in catechin (mg/g)***	***TFC in quercetin (mg/g)***	***TTC in catechin (mg/g)***
Piper betle petroleum ether	31.25	29.58	13.33
Piper betle acetone	47.48	46.08	29.33
Piper betle methanol	34.29	31.08	18.67
Leucosyke capitella petroleum ether	1.68	1.08	0.67
Leucosyke capitella acetone	7.19	6.83	5.33
Leucosyke capitella methanol	2.26	1.83	1.33

3.2 Total Flavonoid Content

The total flavonoid content (TFC) of *Piper betle* ranged from 29.58 to 46.08 mg/g (Table 2) was determined at 100 ppm from standard calibration curve of quercetin (Figure 2). The *Piper betle* extracted with acetone of 46.08 mg/g contain significantly higher flavonoid than the corresponding *Piper betle* extracted with methanol of 31.08 mg/g and petroleum ether of 29.58 mg/g.

The total flavonoid content of *Leucosyke capitella* ranged from 1.08 to 6.83 mg/g (Table 2). The *Leucosyke capitella* extracted with acetone of 6.83 mg/g contain significantly higher flavonoid than the corresponding *Leucosyke capitella* extracted with methanol of 1.83 mg/g and petroleum ether of 1.08 mg/g.

This shows that acetone extracted more flavonoid compound compare to methanol and petroleum ether. These again suggested that acetone is more competitive than methanol to extract polar flavonoid compound for *Piper betle* and *Leucosyke capitella.* Result shows that at any specific types of solvent used in extraction, *Piper Betle* has higher amount of flavonoid compound as compared to *Leucosyke capitella.*

3.3 Total Tannin Content

The total tannin content (TTC) of *Piper betle* ranged from 13.33 to 29.33 mg/g (Table 2) was determined at 100 ppm from standard calibration curve of catechin. The *Piper betle* extracted using acetone had the highest total tannin content of 29.33 mg/g, followed by methanol of 18.67 mg/g and petroleum ether of 13.33 mg/g.

The total tannin content of *Leucosyke capitella* ranged from 0.67 to 5.33 mg/g (Table 2). The *Leucosyke capitella* extracted using acetone had the highest total tannin content of 5.33 mg/g, followed by methanol of 1.33 mg/g and petroleum ether of 0.67 mg/g.

Again, acetone seems to show better extraction capability compare to methanol and petroleum ether and *Piper betle* had more tannin compound than *Leucosyke capitella.*

The comparison of total phenolic, flavonoid and tannin contents of *Piper betle* and *Leucosyke capitella* at Figure 1. From that graph it is highly suggested that the best solvent to extract phenolic, flavonoid and tannin will be acetone. Also, the *Piper betle* contain more phytochemical than *Leucosyke capitella.*

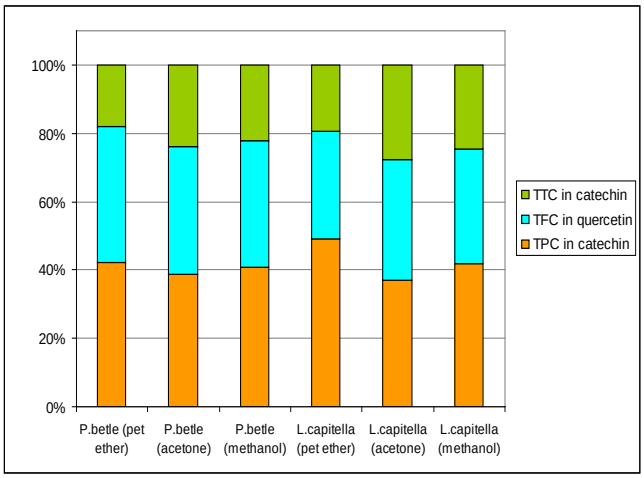

Fig. 1 Total phenolic, flavonoid and tannin contents in *Piper betle* and *Leucosyke capitella*

In this study, the ESR spectrometry assay has been used to measure the free radical scavenging activity of the antioxidant against DPPH radical. Vitamin C was used as a reference antioxidant and screened for comparison purposes

with the synthesised flavanones. Positive DPPH test suggests that the tested compounds are free radical scavengers.

An ESR signal is directly proportional to the number of radicals present. The 0.25 mM DPPH radicals give a typical ESR spectrum as shown in Figure 1.0. The peaks height will be reduced when an antioxidant was added to the ethanolic DPPH solution. The radical scavenging activity of flavanones was expressed by means of SC_{50} which represent the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%.

3.4 Antioxidant Activity

Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The DPPH radical scavenging activity (Figure 2 and 3) decreased in the following order: Quercetin > *Piper betle* methanol > Catechin > *Piper betle* acetone > Vitamin E > *Leucosyke capitella* acetone > BHT > *Leucosyke capitella* methanol > *Piper betle* petroleum ether > *Leucosyke capitella* petroleum ether.

Based on Figure 4, *Piper betle* had higher antioxidant activity than *Leucosyke capitella*. These findings support the results obtained from the total phenolic, flavonoid and tannin content that *Piper betle* plants showed higher content than *Leucosyke capitella* plants. Therefore, the ability to scavenge the DPPH radical for *Piper betle* is better than *Leucosyke capitella*. However, the scavenging of radical efficiency of *Piper betle* plant is less than quercetin antioxidant.

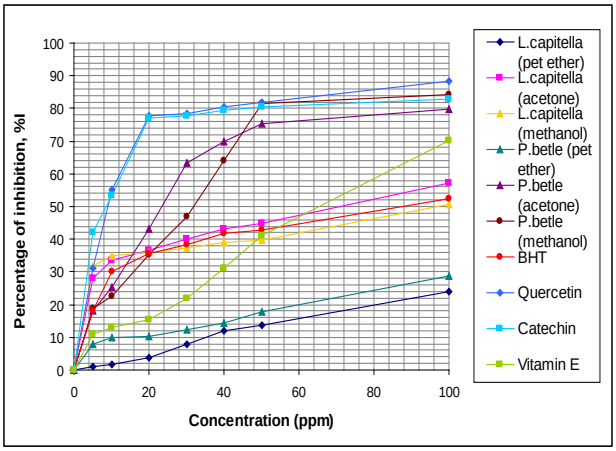

Fig. 2 Percentage of inhibition for BHT, Quercetin, Catechin, Vitamin E, *Piper betle* and *Leucosyke capitella* (Line graph)

The half maximal inhibitory concentration or the concentration of substance that provides 50 % inhibition to certain reaction, IC_{50} is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. From Figure 5, the IC_{50} of *Piper betle* and *Leucosyke capitella* extracted with petroleum ether can not be determined within 0 to 100 ppm concentration of sample. This possibly due to the polarity of solvent, which is the petroleum ether, is lower and only extracted non-polar compound. Thus, the non-polar compound which extracted using petroleum ether is not strong antioxidant because they did not reach steady state to react with DPPH or not enough ionization to donate hydrogen or electrons to quench DPPH radicals. The IC_{50} of the samples decreased in the following order of *Leucosyke capitella* methanol > *Leucosyke capitella* acetone > *Piper betle* methanol > *Piper betle* acetone.

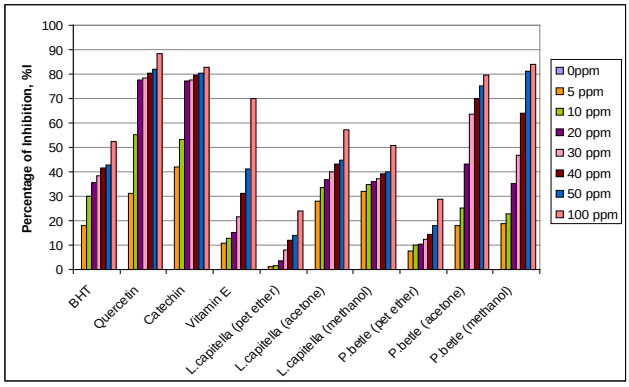

Fig. 3 Percentage of inhibition for BHT, Quercetin, Catechin, Vitamin E, *Piper betle* and *Leucosyke capitella* (Bar graph)

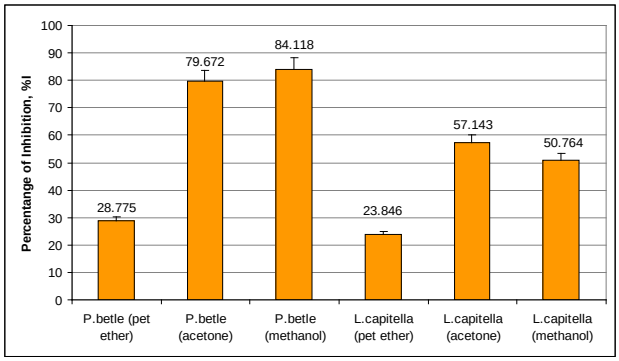

Fig. 4 Percentage of inhibition for *Piper betle* and *Leucosyke capitella* at 100 ppm concentration

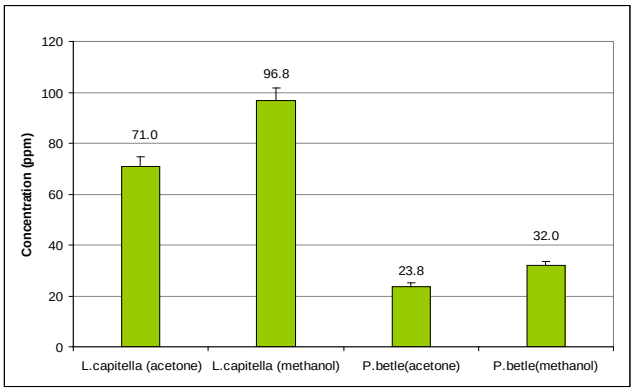

Fig. 5 IC_{50} values for *Piper betle* and *Leucosyke capitella*

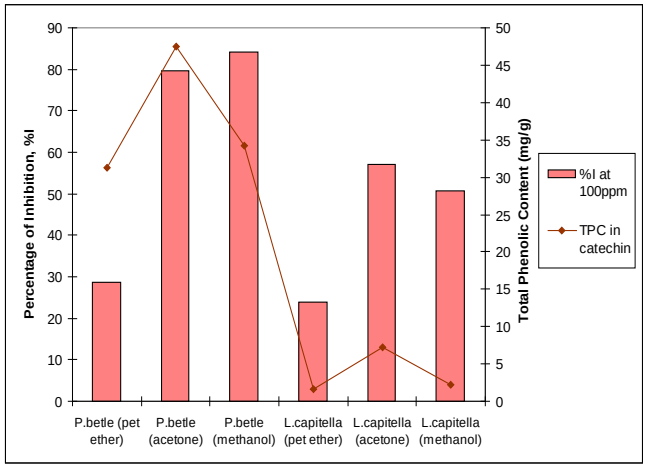

Fig. 6 Percentage of inhibition and total phenolic content for *Piper betle* and *Leucosyke capitella*

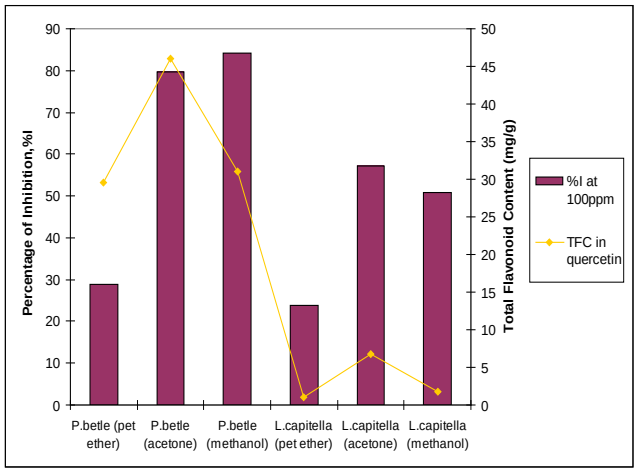

Fig. 7 Percentage of inhibition and total flavonoid content for *Piper betle* and *Leucosyke capitella*

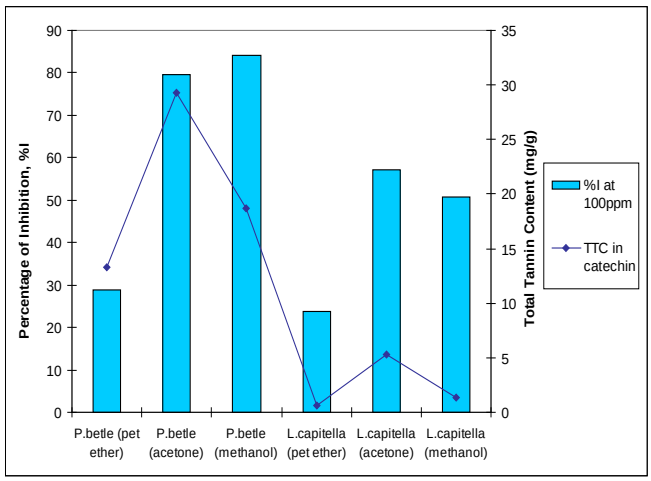

Fig. 8 Percentage of inhibition and total tannin content for *Piper betle* and *Leucosyke capitella*

Furthermore, the percentage of inhibition and total phenolic, flavonoid and tannin contents have similar trend except for the *Piper betle* in methanol, because of the total phenolic, flavonoid and tannin contents decreases but the percentage of inhibition increases compare with *Piper betle* in acetone (Figure 6, 7 and 8).

4. CONCLUSION

The results obtained in this study demonstrate that *Piper betle* and *Leucosyke capitella* contain phytochemical such as phenolic, flavonoid and tannin compounds. It also shows that petroleum ether, methanol and acetone extracts of *Piper betle* and *Leucosyke capitella* have the ability to quench free radicals but petroleum ether contain mild antioxidant since the IC_{50} can not be determined between range 0 to 100 ppm. The extractions using acetone is more efficient to extract phenolic, flavonoid and tannin compounds than methanol and petroleum ether for these types of plants. It also found that *Piper betle* contain more antioxidant compound compare to *Leucosyke capitella*. The ability to scavenge radicals for *Piper betle* herbal plant is better than *Leucosyke capitella* plant but lower than quercetin antioxidant standard.

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APPENDIX

Table 1 Percentage of inhibition for *Piper betle* and *Leucosyke capitella*

Sample	***Percentage of Inhibition, %I at***						
	5 ppm	**10 ppm**	**20 ppm**	**30 ppm**	**40 ppm**	**50 ppm**	**100 ppm**
P. betle pet ether	7.768	10.066	10.394	12.363	14.333	17.943	28.775
P. betle acetone	18.033	25.246	43.060	63.497	69.945	75.301	79.672
P. betle methanol	18.729	22.673	35.268	46.878	63.965	81.380	84.118
L. capitella pet ether	1.099	1.758	3.736	5.022	11.868	13.846	23.846
L. capitella acetone	28.026	33.479	36.641	40.022	43.184	44.820	57.143
L. capitella methanol	31.987	34.825	36.026	37.227	39.192	42.249	53.384

Table 2 IC_{50} values for *Piper betle* and *Leucosyke capitella*

Sample	***IC_{50} (ppm)***
Piper betle acetone	23.8
Piper betle methanol	32.0
Leucosyke capitella acetone	71.0
Leucosyke capitella methanol	96.8

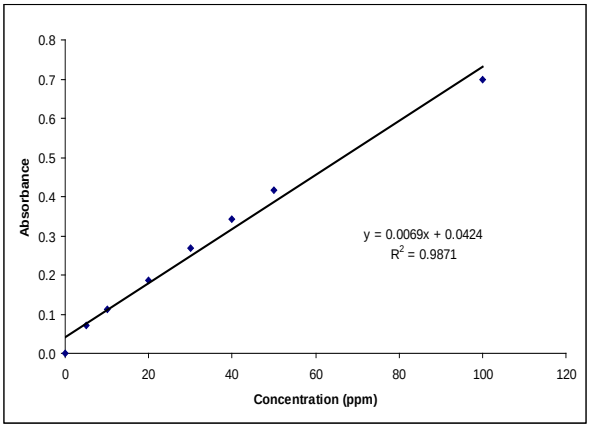

Fig. 1 Standard calibration curve of catechin for determination of total phenolic content

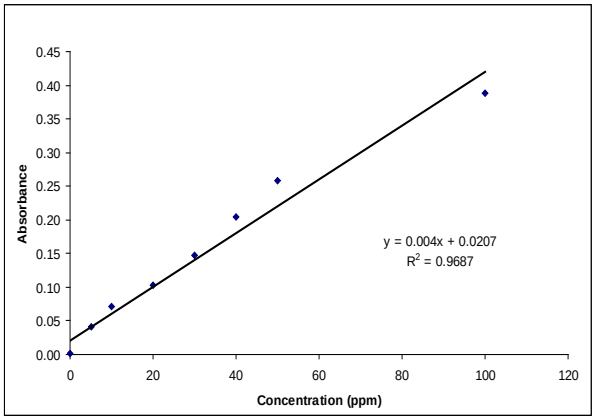

Fig. 2 Standard calibration curve of quercetin for determination of total flavonoid content

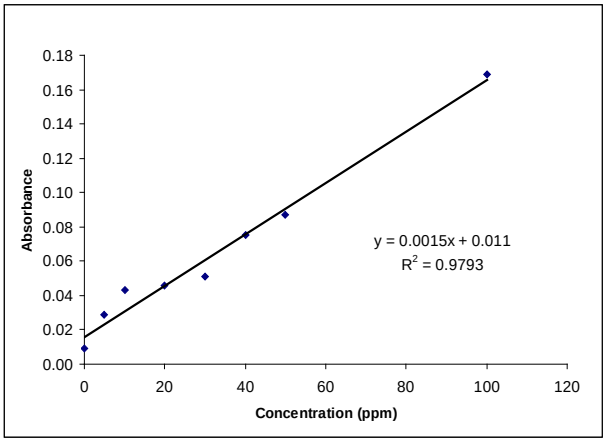

Fig.3 Standard calibration curve of catechin for determination of total tannin content