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## Comparison between maceration and microwave extraction techniques of strawberry fruit (*fragaria sp*) and antioxidant activity test

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# Comparison between maceration and microwave extraction techniques of strawberry fruit (*fragaria sp*) and antioxidant activity test

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**Abstract.** Extraction of strawberry fruit (*Fragaria sp*) by maceration and microwave and antioxidant activity test was studied. The objectives of this research are (1) to compare the maceration and microwave extraction techniques, and (2) to determine the antioxidant activity of strawberry fruit extract. The research steps consist of sample preparation, maceration and microwave extraction with 96% ethanol solution, phytochemical screening test and antioxidant activity test with DPPH method. The yield of extraction of 5.77%, 2.12%, 1.55% and 2.58% is achieved at 24 hours maceration and 3, 5 and 7 min microwaves, respectively. The phytochemical screening result shows that the strawberry fruit ethanol extract contains tannins, flavanoids, alkaloids, and saponins compounds. The identification result of flavanoids compounds by UV-Vis spectrophotometer reveals that the strawberry fruit ethanol extract is interpreted to contain isoflavones compounds. The FTIR spectra displays the existence of specific function groups of flavanoids compound such as OH, C-O alcohol, C=C aromatic, C-H aromatic, C-H alifatic, C=O and C-O ether. Antioxidant activity test by DPPH method reveals that strawberry fruit ethanol extract at 24 h maceration and 3, 5 and 7 min microwave containing IC<sub>50</sub> of 50.61 ppm and 67.97, 118.45 and 61.42 ppm, respectively. Moreover, LC-MS-MS analysis indicates the presence of isoflavones compounds peak i.e. formononetin and daidzin.

## 1. Introduction

Indonesia is known as a country with biodiversity abundance. Indonesia has a tropical climate that support to be host of the development of medicinal plants cultivation. One of the many crops grown in Indonesia is strawberry, especially in West Java. Strawberry fruit is one of sources of bioactive compounds, ascorbic acid, anthocyanin, phenol compounds and has antioxidant property [1,2].

Strawberry (*Fragaria sp*) is a fruit plant with high economic value with sour taste, sweet and fresh. Strawberry fruit contains vitamin C, rich in fiber, folic acid, potassium and antioxidants. The content of strawberry can be used as an alternative to improve heart health, reduce the risk of cancer and beneficial to health [3,4]. Strawberry is rich in color pigments. The red color of strawberry is caused by anthocyanin and has antioxidant properties. Strawberry have a high antioxidant content compared to other fruits and vegetables, so strawberry can be used to overcome the problem of free radical diseases such as cancer, stroke and the aging process. Strawberry also contain low sugar so it is suitable for diabetic diet and arthritis. Due to its high antioxidant, this fruit can also be used to smooth and brighten the skin [5]. The antioxidant substances in strawberry can serve to neutralize free radical compounds and inhibit oxidative processes [6]. Various extraction techniques with various solvents have been performed to extract the antioxidant active substances in strawberry. In this study, we compared the extraction techniques i.e. between maceration and microwave techniques to extract the

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active substances and to test the antioxidant activity of the strawberry fruit extract. Microwave technique showed advantages in terms of high extraction efficiency and antioxidant activity of extract within shortest extraction time [7]. The solvent used in the extraction process is ethanol. The antioxidant activity test method applied in this study is DPPH method (2,2- *Diphenyl-1-Picrylhydrazyl*). The DPPH method is easy to use, fast, thorough enough and sensitive to evaluate the antioxidant activity of natural material compounds [8].

## 2. Methods

### 2.1 Instruments and materials

The experiment was performed by means of a set of glassware, blender, cuvet, rotary evaporator, microwave, UV-Vis Spectrophotometry, FTIR and LC-MS/MS. The materials include strawberries, ethanol, methanol, FeCl<sub>3</sub> 1%, concentrated HCl, Mg powders, Mayer reagent, Wagner reagent and Dragendorff reagent, aquadest, NaOH, AlCl<sub>3</sub>, CH<sub>3</sub>COONa, H<sub>3</sub>BO<sub>3</sub> and DPPH (2,2- *Diphenyl-1-Picrylhydrazyl*).

### 2.2 Sample Preparation

Strawberries were obtained directly from Inggit Strawberry orchards in Banyuroto, Sawangan, Magelang in Central Java. The samples were washed with water, dried and blended until they become a refined powder, which were ready for the extraction process.

### 2.3 Maceration Extraction Method

Strawberry powder was macerated for 2 days (M extract). Sample observation was done every 6 hours. The filtrat was then filtered, collected and evaporated with a vacuum rotary evaporator at 37°C until a viscous extract obtained.

### 2.4 Microwave Extraction Method

Sample A was treated by microwave with power 100 watts with time variation 3 (WA), 5 (WB) and 7 (WC) minutes. Samples of WA, WB and WC were filtered and evaporated with vacuum rotary evaporator at 37°C until obtaining viscous extract.

### 2.5 Testing of strawberry fruit extract includes

Phytochemical screening tests include tannins, flavanoids, alkaloids, and saponins tests. Identification of flavonoid compounds used shift reagents and UV-Vis Spectrophotometry [9]. Identification of compounds of strawberry extract was tested using FTIR. Antioxidant activity test was performed by means of DPPH method. Identification group of compound structure of strawberry extract was done by using LC-MS-MS.

## 3. Results and discussion

This study was aimed to compare the maceration and microwave techniques for the extraction of strawberries and antioxidant test of strawberry extract. The yield of extraction of 5.77%, 2.12%, 1.55% and 2.58% is achieved at 24 hours maceration and 3, 5 and 7 min microwaves, respectively. It means that, the yield of the maceration technique is greater (5.77%) than that of the microwave extraction technique (2.58%) using 96% ethanol. The effectiveness of an extraction process is influenced by the type of solvent used as a filter, the particle size of the sample, the technique and duration of extraction. Maceration technique performed for 2 days so that the longer the extraction time, the more active substances that can be extracted to achieve the balance of diffusion between solvent and solute. The advantages of microwave extraction process has a relatively short time only 3-7 minutes, while the maceration extraction takes 2 days [7].

### 3.1. Phytochemical Screening

Phytochemical screening is a qualitative test of chemical compounds in plants. Phytochemical screening was performed to determine the class of active compounds or secondary metabolite compounds contained in extract of strawberry fruit. It is determined by looking at the color change reaction that occurred after the addition of a reagent used. The results of phytochemical tests shown that the extract contained tannin, flavanoids, alkaloids, and saponin (Table 1).

**Table 1.** Results of phytochemical screening test of strawberry fruit extract

No.	Compounds group	Reagent	Color	Result
1.	Tannin	FeCl <sub>3</sub> 1%	Blackish green	+
2.	Flavonoids	Mg + HCl solid	Red Blood	+
		Dragendrof	Orange	+
3.	Alkaloids	Mayer	White yellowish	+
		Wagner	Reddish brown	+
4.	Saponins	Aquadest	Shaped foam	+

### 3.2. Identification of flavanoid compounds by UV-Vis Spectrophotometers

Table 2 shows the UV VIS wavelength before and after reactions with Shift reagent. Based on the Table 2, band I and band II of all samples were interpreted to contain different flavonoid compounds. This results indicated to the presence of methylation and glycosylation which is particularly present in C-3, C-5, C-7 and C-4 'hydroxyl, which results in a lower shift [9].

#### 3.2.1. Determination of flavanoid compound structure from M sample

Based on Table 2, after addition of the 2M NaOH solution, the M solution was turned to yellow and underwent a leftward hypochromic shift due to the ionization of the alkaline-sensitive hydroxyl group. The shift was occurred in band I of 10 nm and band II of 20 nm. It can be seen that band I and band II shift but does not show the presence of hydroxy group at flavonoid core.

After addition of 5% AlCl<sub>3</sub> solution, the M solution color changed to yellow and causes a 10 nm shift of bathochromic to the band II indicating that it is likely to be suspected in the C-5 position of the OH group present in the type of flavonoid group of isoflavones, whereas in the absence of the band I shift which means there is no possibility of a hydroxy group at the flavonoid core [10].

**Table 2.** UV VIS wavelength of the extract solution before and after reaction with shift reagent.

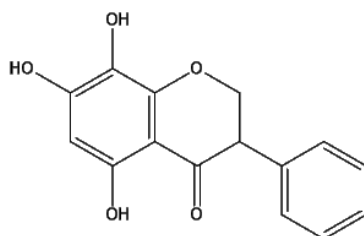
Sample	Wavelength	Extract + CH <sub>3</sub> OH	Extract + CH <sub>3</sub> OH + NaOH	Extract + CH <sub>3</sub> OH + AlCl <sub>3</sub>	Extract + CH <sub>3</sub> OH + CH <sub>3</sub> COONa	Extract + CH <sub>3</sub> OH + CH <sub>3</sub> COONa + H <sub>3</sub> BO <sub>3</sub>
M	Band I	363	353	<i>n.d</i>	368	378
	Band II	263	243	273	263	270
WA	Band I	362	343	<i>n.d</i>	380	381
	Band II	260	279	273	270	263
WB	Band I	363	343	<i>n.d</i>	<i>n.d</i>	382
	Band II	274	245	273	273	268
WC	Band I	363	343	384	<i>n.d</i>	384
	Band II	260	283	272	277	263

n.d = not detected

**Table 3.** Interpretation of wavelength changes of M extract solution with the addition of Shift reagents.

Treatment	Wavelength (nm)		Shift (nm)		Substitution
	Band I	Band II	Band I	Band II	
Extract + CH <sub>3</sub> OH	363	263	-	-	-
Extract + CH <sub>3</sub> OH + NaOH	353	243	-10	-20	-
Extract + CH <sub>3</sub> OH + AlCl <sub>3</sub>	<i>n.d</i>	273	-	+10	5-OH (isoflavones)
Extract + CH <sub>3</sub> OH + CH <sub>3</sub> COONa	368	263	+5	Tetap	7-OH (isoflavones)
Extract + CH <sub>3</sub> OH + CH <sub>3</sub> COONa + H <sub>3</sub> BO <sub>3</sub>	378	270	+15	+7	<i>o</i> -diOH in ring B, <i>o</i> -diOH in ring A (6,7 atau 7,8)

Based on data interpretation of maximum wavelength change by using addition of shift reagent, the extract of strawberry fruit possibly contains isoflavones. It can be proven by the formed spectrum which exhibits the characteristics of isoflavones with the possible presence of hydroxy groups in C-5, C-7 and C-8. The structure of the isoflavone trihydroxy compound is shown in Figure 1.



**Figure 1.** Structure of the 5,7,8-Trihydroxy Isoflavone

### 3.2.2. Determination of flavanoid compound structure from WA sample

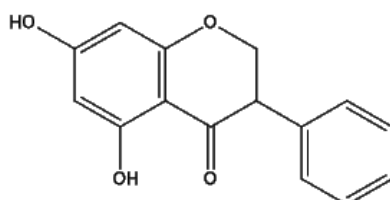
The result of UV-Vis spectra of WA sample with addition of 2M NaOH solution are hypochromic shift in band I of 19 nm and bathochromic shift in band II of 19 nm. It can be seen that in band I and band II has shift but does not show the presence of hydroxyl group at flavonoid core. Addition of AlCl<sub>3</sub> 5% solution results a 13 nm shift in bathochromic of band II indicates that C-5 is suspected to be a 5-OH group. Whereas in band I does not experience a shift which implies that it does not show the presence of a hydroxyl group at the flavonoid core. Addition of the CH<sub>3</sub>COONa solution causes a bathochromic shift in band I of 18 nm and band II of 10 nm indicating that it is likely to be suspected in position C-7 of the OH group present in the type of flavonoid class of isoflavones.

Addition of shift reagents CH<sub>3</sub>COONa and H<sub>3</sub>BO<sub>3</sub> results a shift in the band I of 19 nm indicating that the possibility of *o*-dihydroxyl in ring B, while the shift in the band II of 3 nm, although shifted but did not show the presence of hydroxy group at the flavonoid core (Table 4).

Based on interpretation of data in Table 4, the compound contained in WA extract possibly is isoflavones. It can be seen from the formed spectrum is characteristic of isoflavones with the presence of hydroxy group on C-5 and C-7. The structure of the isoflavone 5,7-dihydroxy compound is shown in Figure 2.

**Table 4.** Interpretation of wavelength changes of WA extract by the addition of shift reagents.

Treatment	Wavelength (nm)		Shift (nm)		Alleged Substitution
	Band I	Band II	Band I	Band II	
Extract + CH <sub>3</sub> OH	362	260	-	-	-
Extract + CH <sub>3</sub> OH + NaOH	343	279	-19	+19	-
Extract + CH <sub>3</sub> OH + AlCl <sub>3</sub>	<i>n.d</i>	273	-	+13	5-OH (isoflavones)
Extract + CH <sub>3</sub> OH + CH <sub>3</sub> COONa	380	270	+18	+10	7-OH (isoflavones)
Extract + CH <sub>3</sub> OH + CH <sub>3</sub> COONa + H <sub>3</sub> BO <sub>3</sub>	381	263	+19	+3	o-dihydroxyl in ring B



**Figure 2.** Structure of the Isoflavone 5,7-dihydroxy

### 3.2.3. Determination of flavanoid compound structure of WB extract

Table 5 shows the wavelength change of WB extract solution before and after reaction with Shift reagent. Reaction with 2M NaOH results a hypochromic shift in band I of 20 nm and a band of II of 29 nm. After addition of 5% AlCl<sub>3</sub> solution and CH<sub>3</sub>COONa solution, there are no significant shifting of wavelength. it indicates that possibly there no hydroxyl group in the flavonoid core. Reaction with CH<sub>3</sub>COONa and H<sub>3</sub>BO<sub>3</sub> solution results a bathochromic shifting in band I of 19 nm. It is suspected that the o-dihydroxyl group in ring B, where as in band II, there is no significant shifting. Based on interpretation of the wavelength change, the WB extract possibly contains isoflavones. The isoflavone structure is shown in Figure 3.

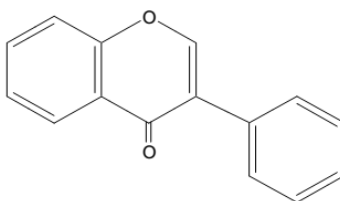
### 3.2.4. Determination of flavanoid compound structure of WC extract

Table 6 shows the wavelength change of WC extract solution before and after reaction with Shift reagent. Reaction with 2M NaOH results a hypochromic shift in band I of 20 nm and a bathochromic band II of 23 nm. Reaction of WC extract with of 5% AlCl<sub>3</sub> solution results a shift in band I of 21 nm indicating the possibility of suspected o-dioxide in ring A. However, in band II shifts 12 nm, reveals the presence of OH group at position C-5. Reaction with CH<sub>3</sub>COONa and H<sub>3</sub>BO<sub>3</sub> solution results a

bathochromic shifting in band I of 17 nm indicating the presence of OH group at position C-7. Addition of  $\text{CH}_3\text{COONa}$  and  $\text{H}_3\text{BO}_3$  solution to the WC extract solution causes a shift of bathochromic in band I of 21 nm indicating that the presence of o-diOH group in ring B. However, there is no significant shifting on band II.

**Table 5.** Interpretation of wavelength changes of WB extract by the addition of Shift reagents.

Treatment	Wavelength (nm)		Shift (nm)		Alleged Substitution
	Band I	Band II	Band I	Pita II	
Extract + $\text{CH}_3\text{OH}$	363	274	-	-	-
Extract + $\text{CH}_3\text{OH}$ + NaOH	343	245	-20	-29	-
Extract + $\text{CH}_3\text{OH}$ + $\text{AlCl}_3$	<i>n.d</i>	273	-	-1	-
Extract + $\text{CH}_3\text{OH}$ + $\text{CH}_3\text{COONa}$	<i>n.d</i>	273	-	-1	-
Extract + $\text{CH}_3\text{OH}$ + $\text{CH}_3\text{COONa}$ + $\text{H}_3\text{BO}_3$	382	268	+19	-6	o-diOH in ring B

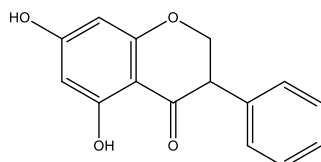


**Figure 3.** Structure of the Isoflavone

**Table 6.** Interpretation of wavelength changes of WC extract by the addition of Shift reagents.

Treatment	Wavelength (nm)		Shift (nm)		Alleged Substitution
	Band I	Band II	Band I	Pita II	
Extract + $\text{CH}_3\text{OH}$	363	260	-	-	-
Extract + $\text{CH}_3\text{OH}$ + NaOH	343	283	-20	+23	
Extract + $\text{CH}_3\text{OH}$ + $\text{AlCl}_3$	384	272	+21	+12	o-diOH in ring A, 5-OH (isoflavones)
Extract + $\text{CH}_3\text{OH}$ + $\text{CH}_3\text{COONa}$	<i>n.d</i>	277	-	+17	7-OH (isoflavones)
Extract + $\text{CH}_3\text{OH}$ + $\text{CH}_3\text{COONa}$ + $\text{H}_3\text{BO}_3$	384	263	+21	+3	o-diOH in ring B

Based on interpretation of wavelength change using shift reagent, the WC extract possibly contains isoflavones. It can be seen from the formed spectrum is characteristic of isoflavones with the presence of dihydroxy group on C-5 and C-7 (Figure 4).



**Figure 4.** Structure of the Isoflavone 5,7-dihydroxy.

### 3.3. IR spectrum of Strawberry extract

The four extracts of the strawberries were then analyzed using FTIR which aimed to identify the functional group. Identification of compounds using FTIR to further strengthen the presence of flavonoid compounds. Based on infrared spectrum of strawberries extracts in Figure 5, it shows that there are several functional groups in the crude extract. Absorption at  $3271.48\text{ cm}^{-1}$  indicates the presence of -OH groups in aliphatic and aromatic groups. It is a result of vibration of intramolecular hydrogen bonds which is supported by the vibration of CO alcohol bending on  $1051.09\text{ cm}^{-1}$ . Both of these absorption peaks indicate that there is an OH group of alcohols attached to the carbon atoms. This assumption is reinforced by the presence of C = C aromatic absorption at  $1620.67\text{ cm}^{-1}$ . The strong absorption peaks of the aromatic C - H bend are present at the  $816.81\text{ cm}^{-1}$ . The  $2932.52\text{ cm}^{-1}$  wave number is suspected the existence of alpha C-H. A strong absorption band of  $1714.71\text{ cm}^{-1}$  indicates the presence of a carbonyl group (C = O), suspected as a common feature of the flavonoid group compound. The absorption peak of the vibration of the C-O ether group ( $1101.56\text{ cm}^{-1}$ ). Based on the assumption reinforced by the presence of functional groups such as OH, CO alcohol, C = C aromatic, CH aromatic, CH aliphatic, C = O and CO ether functional groups possessed by flavonoid compounds [11,12]. Based on data of UV-Vis and infrared spectrum, the strawberries extract possibly contains isoflavone compounds with the possibility of hydroxy group at C-5, C-7 and C-8 on the A ring.

### 3.4. Antioxidant Activity of Strawberry Extract

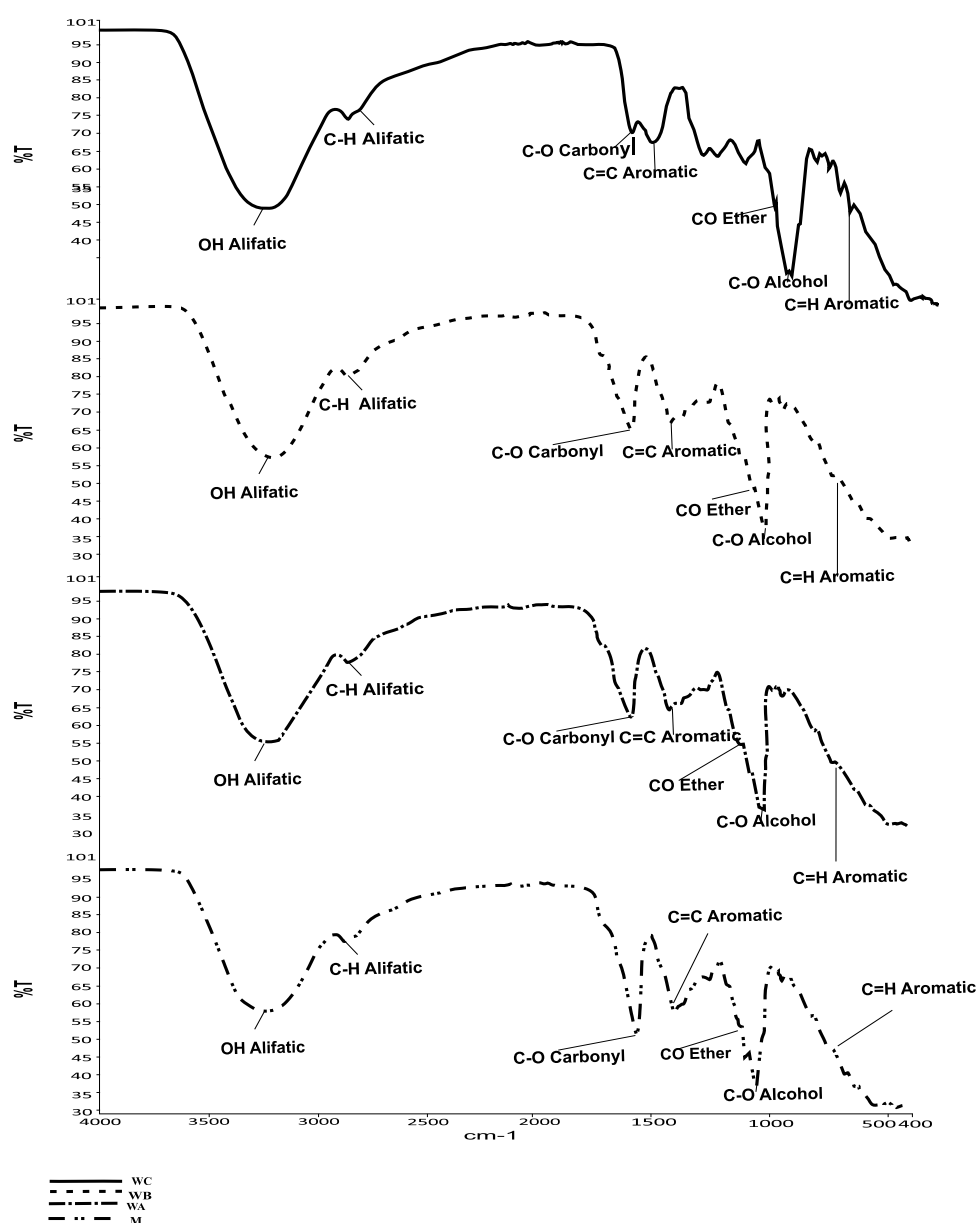
Antioxidant activity of crude strawberry extract was assessed using DPPH method [13]. The DPPH method uses 2,2 diphenyl-1-picrylhydrazyl as a stable free radical compound. This method is the commonly used antioxidant assay for plant extract. Four crude strawberries extract, M, WA, WB, and WC extract were assessed to determine its antioxidant activity. Based on  $IC_{50}$  value, M extract has a very strong activity (50.6 ppm). Where as WA (68.0 ppm) and WC (61.4 ppm) extracts have strong activities, and WB has a medium antioxidant activity (118.5 ppm) (Table 7). This result reveals that the maceration technique is the suitable technique to extract antioxidant substance from strawberry fruit compare to microwave technique. The maceration technique was conducted in room temperature whereas using the microwave technique resulted a higher temperature in extraction process. Possibility, a higher temperature could be damage the active compounds.

### 3.5 Identification of antioxidant activity compound structure using LC-MS-MS [14]

The quadrupole MS / MS Triple Q mass spectrometer with an electrospray (ESI) mode and quantification of the Selected Reaction Monitoring (SRM) method were employed for identification of flavonoids in *Fragaria* sp. The SRM mode can provide high specificity, sensitivity and by the structures of unknown of flavonoids may be accessed based on the  $m/z$  of both ion and fragment ion obtained through mass transition [15,16]. Moreover, the absorption spectral data were compared with that reported in the literature. Fragment ions  $[M-H]^+$  was identified at  $m/z$  253-223, the parent ion  $[M]^+$  at



$m/z$  267 and absorption maximum at 263 nm obtained due to there are of hidroxyl groups from isoflavon that revealing the compound to be **formononetin**. The presence of two hexose moieties linked to a luteolin aglycone in the structure for peak 3 was identified by the fragment ions at  $m/z$   $[M-H]^+$  at  $m/z$  294.5-295.5. The parent ion  $[M]^+$  at  $m/z$  415 and  $\lambda$  max at (368, 353 nm bond 1 and 263, 243 nm bond 2) obtained was tentatively identified as a derivate of **daidzin** by comparison with the literature [17].



**Figure 5.** Infrared Spectrum of strawberry extract.

**Table 7.** The strength of antioxidants based on IC<sub>50</sub> value

Method	IC <sub>50</sub> (ppm)	Very strong (< 50 ppm)	Strong (50-100 ppm)	Medium (100-150 ppm)	Weak (150-200 ppm)
M	50.6	√			
WA	68.0		√		
WB	118.5			√	
WC	61.4		√		

#### 4. Conclusions

Phytochemical screening test indicates the presence of compounds tannin, flavonoids, alkaloids and saponins in crude strawberry extract. The identification result using LC-MS-MS shows the existence of isoflavones compound peak i.e. formononetin and daidzin. The extraction techniques comparison result of strawberry fruit ethanol extract shows the highest yield and the best antioxidant activity with maceration techniques. This shows that the antioxidant activity of strawberry fruit extract with maceration technique is more active than the microwave technique. The maceration technique is the suitable extraction technique to achieve the highest antioxidant activity of Strawberry extract.

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