Jurnal Teknologi

Full Paper

# EFFECT OF pH ON ADSORPTION OF ORGANIC ACIDS AND PHENOLIC COMPOUNDS BY AMBERLITE IRA 67 RESIN

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**Article history**
Received
*29 March 2018*
Received in revised form
*9 August 2018*
Accepted
*1 September 2018*
Published online
*15 December 2018*

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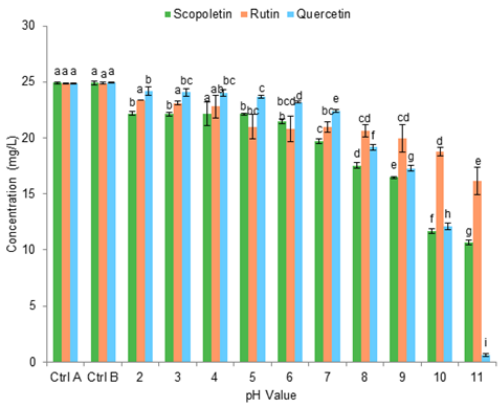
## Graphical abstract




## Abstract

Adsorption of model solution containing organic acids (octanoic and hexanoic acid) and phenolic compounds (rutin, scopoletin and quercetin) on a weak base anion exchange Amberlite IRA 67 resin was studied in a model system. This research was carried out to understand the effect of pH on single and multicompounds studied for its further use in actual system (fruit system). It was shown that the pH dependence of adsorption capacity of weak base anion exchange Amberlite IRA 67 resin had similar increasing trend on organic acids (hexanoic and octanoic acid) and phenolic compounds (rutin, scopoletin and quercetin) studied as pH values increased. In single solution of each phenolic compound, it was observed that total phenolic content (TPC) and antioxidant activity (FRAP and DPPH) gave highest values closer to neutral pH regime. The pH dependence of adsorption capacity in multicompounds solution also showed similar trend for organic acid compounds. Similar trend was also found in multicompounds solution of phenolic compounds in total phenolic content (TPC) and antioxidant activity (FRAP and DPPH). The findings obtained in this study will help to gain better understanding of the complex mechanisms of ion exchange resin and adsorption process involving multicompounds system.

*Keywords:* Adsorption, ion exchange resin, hexanoic acid, octanoic acid, phenolic compounds, pH

## Abstrak

Penjerapan larutan model iaitu asid organik (asid oktanoik dan asid heksanoik) dan sebatian fenolik (rutin, skopoletin dan kuersetin) pada resin penukar ion bes lemah Amberlite IRA 67 dikaji di dalam sistem model. Kajian ini dijalankan untuk memahami kesan pH pada larutan sebatian tunggal dan sebatian multi untuk diaplikasikan di dalam sistem sebenar (sistem buah). Hasil kajian ini menunjukkan kebergantungan pH pada kapasiti penjerapan resin penukar ion bes lemah Amberlite IRA 67 mempunyai tren yang meningkat pada asid organik asid organik (asid oktanoik dan asid heksanoik) dan sebatian fenolik (rutin, skopoletin dan kuersetin) apabila nilai pH ditingkatkan. Dalam larutan tunggal bagi setiap sebatian fenolik,pemerhatian menunjukkan kandungan fenolik jumlah (TPC) dan aktiviti antioksida (FRAP dan DPPH) memberikan nilai bacaan tertinggi menghampiri pH neutral. Kebergantungan pH pada kapasiti penjerapan di

dalam larutan sebatian multi juga menunjukkan tren yang sama untuk sebatian asid organik. Tren yang sama turut diperhatikan pada larutan sebatian multi bagi sebatian fenolik (TPC) dan aktiviti antioksida (FRAP dan DPPH). Penemuan yang diperolehi daripada kajian ini akan membantu untuk lebih memahami mekanisme yang kompleks pada resin penukar ion dan proses penjerapan yang melibatkan sistem sebatian multi.

*Kata kunci:* Penjerapan, resin penukar ion, asid heksanoik, asid oktanoik, sebatian fenolik, pH



## 1.0 INTRODUCTION

*Morinda citrifolia* L. is a Rubiaceous plant widely distributed in many tropical areas [1]. Commonly called noni, it is used traditionally to treat a broad range of diseases reportedly for over 2000 years [2]. About 160 phytochemical compounds have already been identified in *M. citrifolia* plant, and the major micronutrients are phenolic compounds, organic acids and alkaloids [3]. The unpleasant odor of *M. citrifolia* extract was reported to have been contributed by medium chain fatty acids such as decanoic, hexanoic and octanoic acid [4]. Deacidifcation of *M. citrifolia* juice has been attempted by previous researchers using activated charcoal [4]), calcium carbonate [5,6] and fermentation [7]. However, although the unpleasant odor was reduced, antioxidant activities were also reduced. Base anion exchange resin (Amberlite IRA 67) showed promising potential to be used for deacidification while it also gave minimal reduction on antioxidant content [8].

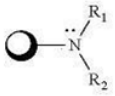
Recently, more attention has been focused on the role of natural antioxidants, in particular, phenolic compounds, which may act both by reducing the content of toxic compounds in foods and by supplying the human body with exogenous antioxidants [9]. Scopoletin is a characteristic phytochemicals in *M. citrifolia* fruits, while rutin and quercetin are bioactive flavonoids [10] commonly found in *M. citrifolia* juice as reported by previous researchers [2,10, 11, 12, 13, 14, 15, 16]. Due to the beneficial role of antioxidants, it is important that deacidification did not reduce the antioxidant activity. The pH of the aqueous solution is an important controlling parameter in any adsorption process, particularly in the adsorption capacity [17]. The pH value can affect the process by affecting the surface change of adsorbent, the degree of ionization and speciation of adsorbate during adsorption [18]. In this model system studied, the solution of organic acids and phenolic compounds were used to stimulate actual noni juice using identical values of pH. The desired condition when adsorption capacity of organic acids is at the lowest while phenolic compound is at the highest can be achieved by manipulating pH of the solution.

## 2.0 METHODOLOGY

### 2.1 Materials

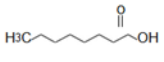
Weak base anion exchanger Amberlite IRA 67 (food grade) (Table 1) and standard of octanoic acid, hexanoic acid, rutin, scopoletin and quercetin were purchased from Sigma Aldrich Corporation (St. Louis, MO, USA). Methanol (purity > 99.9%), HPLC grade was purchased from Fisher Scientific (New Jersey, USA). The molecular structure of organic acids and phenolic compounds were shown in Figure 1.

**Table 1** Chemical and physical properties of resin

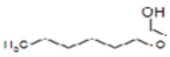
| | **Amberlite IRA 67** |
|---|---|
| Chemical matrix | Crosslinked acrylic gel |
| Functional group | Tertiary amine  |
| Total exchange capacity (eq/L) | ≥ 1.60 (FB form) |
| Physical form | Translucent white spherical beads |
| Particle size (mm) | 0.50 – 0.75 |
| Producer | Sigma Aldrich, Germany |

*Source:* Rohm and Haas

a) Organic acids

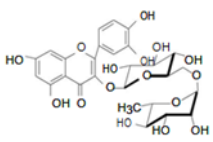


Octanoic acid

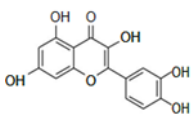


Hexanoic acid

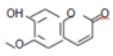
b) Phenolic compounds



Rutin



Quercetin



Scopoletin

**Figure 1** Molecular structure of organic acids and phenolic compounds

### 2.2 Adsorption Studies of Organic Acids and Phenolic Compounds

The adsorption studies were carried out in Erlenmeyer flasks where 0.1 g of the dry anion exchanger and 0.02 L of each samples which were octanoic acid (250 mg/L), hexanoic acid (100 mg/L), rutin (25 mg/L), scopoletin (25 mg/L), and quercetin (25 mg/L), were added without pH adjustment. The flasks were agitated in the orbital shaker (WiseCube, Daihan Scientific, Korea) at a constant speed of 120 rpm for 180 min to achieve equilibrium. Each of the organic acids after equilibrium adsorption was measured using a Gas Chromatography (Agilent, Model HP6890, USA) equipped with Flame Ion Detector (FID). Meanwhile, each of the phenolic compounds was measured using High Perfomance Liquid Chromatography (HPLC). The amount of organic acids and phenolic compounds adsorbed at equilibrium, $q_e$ (mg/g), were calculated as the Equation 1 below:

$$q_e = \frac{C_o - C_e}{w} \times V \quad (1)$$

Where $C_o$ and $C_e$ are the concentrations of the each organic acids and phenolic compounds at the beginning and in the equilibrium, respectively (mg/L); V is the volume of the solution (L); w is the mass of the dry anion exchanger (g).

### 2.3 pH Determination

pH determination were carried out on all samples using (Model PB-10, Sartorius Basic Meter, Germany). The pH meter was calibrated using pH 4.0 and 7.0 buffer. The solution of each compound was stirred before measuring the pH values. The pH was adjusted using dilute NaOH and HCl solutions. The reading was taken at room temperature.

### 2.4 UV-Vis Spectroscopy

UV-Vis absorption spectra of rutin, scopoletin and quercetin were recorded on a spectrophotometer (Shimadzu, UV-2450, Japan) in 4.5 mm based on wavelength of maximum absorbance. The wavelength for rutin was 350 nm and quercetin 370 nm [19], and scopoletin was at 280 nm [20]. Samples were put in quartz cuvette (1 cm x 1 cm x 4.5 cm) at reading were taken at room temperature.

### 2.5 Determination of Organic Acids using GC

Organic acids (octanoic acid and hexanoic acid) were extracted using Gas Chromatography for 10 min according to the previous study done by [21] with some modification. A 1µl sample was inserted immediately using microliter syringe (Hamilton, Model #701, USA) into the injection port of a Gas Chromatography Mass Spectrometry (Agilent, Model HP6890, USA) equipped with Flame Ion Detector (FID) and split injector using an inlet SPME 0.75 mm (Supelco). A capillary column HP-5 (30m x 0.25 i.d., 0.25 µm film thickness, J&W Scientific Pte Ltd, USA) was used.

Nitrogen ($N_2$) was used as carrier gas. Oven temperature was programmed according to the method of [22] with some modifications. Initial temperature was 80°C for 2 min before raised to 80°C at 20°C/min for 1 min, then heated to 100°C at 20°C/min for 1 min. When it reached 100°C, the temperature finally raised to 250°C at 30°C/min and held for 1 min. The gas flow rate was 40 cm/s. The total time for separation for each samples were 10 min. Percentage of peak area were determined by comparing the peak retention time for the standard of octanoic acid with the peak retention time for deacidified samples. The analysis was expressed as concentration (mg/L) obtained from the software.

### 2.6 Determination of Ferric Reducing Assay (FRAP)

FRAP assay was conducted according to the method of [23] with some modification. FRAP reagent was prepared from acetate buffer (0.3 M, pH 3.6), 10 mM TPTZ solution in 0.04 M HCl and 0.02 M iron (III) chloride solution in proportion of 10:1:1 (v/v) respectively. The FRAP reagent was prepared fresh daily and incubated at 37°C in waterbath prior to use. A total of 50 µl samples juice were added to 1.5ml of the FRAP reagent and mixed well. The absorbance was measured at 593 nm using spectrophotometer (Spectronic 200, Madison, WI USA) after 4 min time. Samples were measured in three replications. A standard curve was prepared using a series of standard solution of iron (II) sulphate (200 – 1000 µM). The results were expressed as µmol/ g fresh weight (FW) sample.

### 2.7 Determination of Free Radical Scavenging using DPPH Method

The antioxidant activity of all juices were evaluated through free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [24]. Two ml of 0.1mM DPPH methanolic solution was added into 200 µl of sample juice and 0.8 ml methanol. The mixture was prepared by mixing 2 ml of DPPH and 1 ml methanol. The absorbance was measured at 517 nm using spectrophotometer. Samples were measured in three replications. Percentage of free radical scavenging activity was calculated based on the formula below:

% inhibition of DPPH = [Abs control – Abs sample/ Abs control] x 100.

### 2.8 Determination of Total Phenolic Content

Total phenolic content of noni juice was determined using Folin-Ciocalteau reagents as described by [25]. Samples were inserted into different test tube and

mixed thoroughly with 5ml Folin-Ciocalteau reagent (previously pre-dilute 10 times with distilled water). After 5 min, 4 ml of 7.5% sodium carbonate (Na2CO3) was added and allowed to react for 2 hrs at room temperature. The absorbance was measured at 765 nm using spectrophotometer in three replications. Standard curve of gallic acid solution (0, 10, 25, 50 and 100 ppm) was prepared using the similar procedure. The results were expressed as mg GAE/g FW.

### 2.9 Determination of Phenolic Compounds using HPLC

Methanol (MeOH) and chemical standards of scopoletin, rutin hydrate and quercetin were obtained from Sigma-Aldrich (St. Louis, MO, USA). The standards were accurately weighed and then dissolved in appropriate volume of MeOH/ deionized water to produce corresponding stock standard solutions. Working standard solutions for calibration curves were prepared by diluting stock solutions with MeOH at different concentrations. All stock and working solutions were maintained at 0°C. Deionized water was used throughout. Samples were kept at -20°C before analysis and filtered through a 0.22 µm membrane filter (Iwaki Glass) and injected directly into the HPLC.

The HPLC analysis on phenolic compounds were performed according to the modified method of Analytical HPLC Application 031481, Merck, USA (2008). The system consisted of chromatographic separation performed on a Shimadzu Chromatography 20A with photodiode array detector (PDA), and equipped with Chromolith Performance RP-18 endcapped, Merck, UK (Cat. No. 1.02129). The pump was connected to a mobile phase system composed of two solvents: A; Methanol/ deionized water (2.5: 97.5, v/v) and B; Methanol/ deionized water (50:50, v/v). The mobile phase was programmed consecutively in linear gradient as follows: 0-10 min, 100% A, 0% B; 10-15 min, 65% A, 35% B; 15-20 min, 0% A, 100% B; 20-22 min, 100% A, 0% B; and 22-25 min, 100% A, 0% B. The elution was ran at a flow rate of 2.1 mL/ min at 25 min. The gradient was selected as it afforded a good separation and symmetrical peak shape of target analytes in the HPLC chromatograms. The UV spectra was monitored in the range of 210 to 450 nm for the quantitative analysis. Sample peaks in the chromatograms derived from the photodiode array were integrated at 365 nm. The injection volume was 20 µL for each of the sample and standard solutions. The column temperature was maintained at 30°C. Quantification was based on the peak area measurement. Characterization of the three phenolic compounds were achieved by comparing the HPLC retention time and absorption of target peaks in the samples with those of the standards. Data collection and integration were performed using Shimadzu Lab Solution software.

### 2.10 Statistical Analysis

Three replications were used for all parameters measured. Analysis of the data was analyzed using Excel (Microsoft Inc.) and SAS version 6.12. Statistical tests used were ANOVA and Duncan's Multiple Range test. Data obtained were reported as mean + standard deviation.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Effect of pH on Adsorption Capacity

In this study, the influence of pH on adsorption of hexanoic acid is shown in Figure 2. Control sample A (hexanoic acid solution without resin and agitation) and B (hexanoic acid solution without resin with agitation) gave significantly ($p < 0.05$) lowest adsorption capacity among all treatments, but both are not significantly different to hexanoic acid solution at pH 2 and 3. As pH increased, the adsorption capacity of hexanoic solution increased. Thereafter, the adsorption capacity remained high up to pH 11. The highest ($p<0.05$) adsorption capacity of hexanoic solution was found at pH 10 and 11.

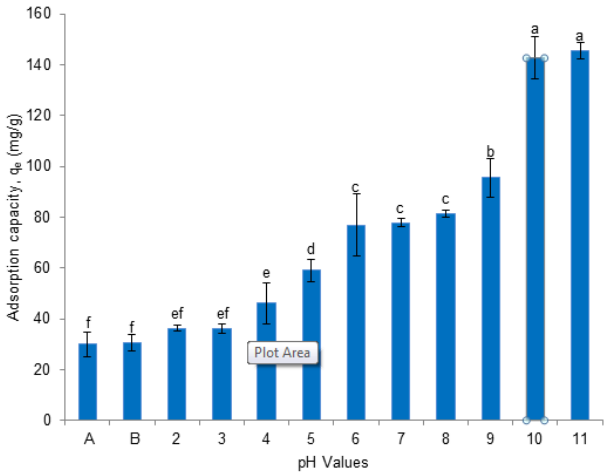



**Figure 2** Effect of pH of hexanoic acid on the adsorption by weakly base ion exchange resin Amberlite IRA 67 (Volume: 20 ml; resin mass: 100 mg; temperature: 303 K; agitation speed: 120 rpm). A: Hexanoic acid solution without resin and agitation, B: Hexanoic acid solution without resin with agitation

Figure 3 shows the effect of octanoic acid solution at different pH on the adsorption of Amberlite IRA 67. As obtained for hexanoic acid solution, there is no significant difference between control samples A and B. However, both control samples gave significantly ($p<0.05$) higher adsorption capacity in octanoic acid solution compared to treated samples with pH modification (pH 2 to 11). The trend for treated octanoic acid solution was similar as in hexanoic acid where there

was an increase of adsorption capacity as the pH values increased. In the octanoic acid solution, it was found that no significant difference between samples of pH 8 to 11. Octanoic acid solution showed significantly ($p<0.05$) the highest adsorption capacity at these pH values compared to other pH studied.

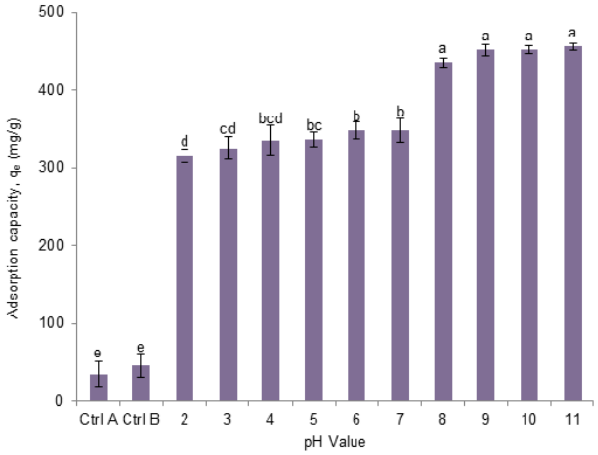



**Figure 3** Effect of pH of octanoic acid on the adsorption by weakly base ion exchange resin Amberlite IRA 67 (Volume: 20 ml; resin mass: 100 mg; temperature: 303 K; agitation speed: 120 rpm). A: Octanoic acid solution without resin and agitation, B: Octanoic acid solution without resin with agitation

The trend of adsorption capacity of these organic acid solutions in single compound were quite similar. Adsorption of organic acids (hexanoic and octanoic acid) on the resin surface can be affected by pH of solution, due to the rate of protonation of the compounds in aqueous solution. The adsorption capacity increases with increasing pH values. It can be explained by the reduction of the positive repulsive interactions between functional groups at high pH. Therefore, the adsorption of low acidic solution is more favoured. A similar phenomenon was reported in the study done by [26] during the adsorption of polyethyleneimine using anion exchange resin.

The effect of pH in the solution on the adsorption capacity, $q_e$ (mg/g) of rutin on Amberlite IRA 67 was studied at a pH range of 2-11 as shown in Figure 4. The experiment was performed with an initial rutin concentration of 25 mg/L, at 303 K with a contact time of 327 min. Control samples A and B gave significantly ($p<0.05$) lowest adsorption capacity among all the treatments, but both are not significantly different.

Agitation rate and time did not affect the concentration of rutin. Rutin solutions were adsorbed by the Amberlite IRA 67 at all pH ranges. This could explain that pH of the solution gives significant effect on biosorption process as mentioned by [27]. At pH 2, adsorption capacity is significantly ($p<0.05$) the lowest compared to other pH studied. It gradually increased with increasing pH values up to pH 11. Rutin solution showed significantly ($p<0.05$) the highest adsorption capacity at pH 11.

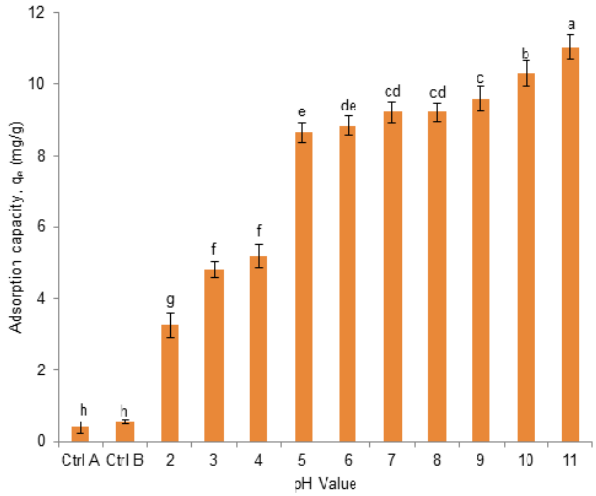



**Figure 4** Effect of pH of rutin on the adsorption by weakly base ion exchange resin Amberlite IRA 67 (Volume: 20 ml; resin mass: 100 mg; temperature: 303 K; agitation speed: 120 rpm). A: Rutin solution without resin and agitation, B: Rutin solution without resin with agitation

Figure 5 shows the effect of pH in the solution on the adsorption capacity, $q_e$ (mg/g) of scopoletin on Amberlite IRA 67 in different pH ranges. Similar to rutin solution, no significant difference between control sample A and B of scopoletin solution but gave significantly ($p<0.05$) the lowest adsorption capacity among all the pH studied. There were no significant difference of scopoletin solution at pH 2 up to pH 5, then increased significantly ($p < 0.05$) to pH 6. At pH 7 and 8, also no significant difference observed until it reached pH 9 to 11 which showed significantly ($p<0.05$) the highest adsorption capacity.

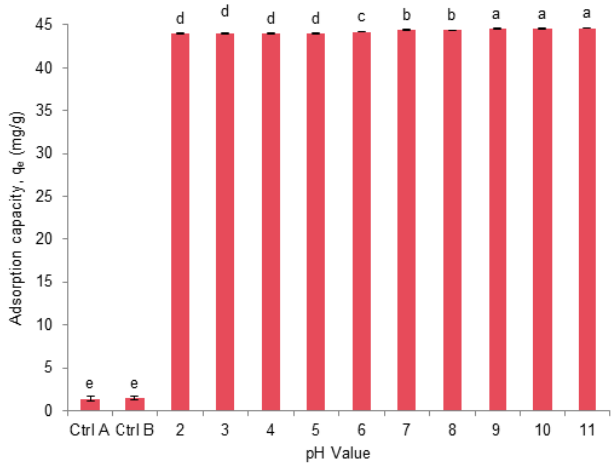



**Figure 5** Effect of pH of scopoletin on the adsorption by weakly base ion exchange resin Amberlite IRA 67 (Volume: 20 ml; resin mass: 100 mg; temperature: 303 K; agitation speed: 120 rpm). A: Scopoletin solution without resin and agitation, B: Scopoletin solution without resin with agitation

Figure 6 displays the effect of pH in the solution on the adsorption capacity, $q_e$ (mg/g) of quercetin on Amberlite IRA 67 in different pH. As can be seen in the figure, no significant difference was observed between control sample A and B. Similar to rutin and scopoletin solution, agitation rate and time did not affect to the adsorption capacity of quercetin solution. However, a significant ($p<0.05$) increase in adsorption capacity of quercetin solution was observed as pH values increase from pH 2 to 4, and from pH 4 to 5, and again started increasing significantly ($p<0.05$) at pH 8. Adsorption capacity of quercetin solution reached the highest adsorption capacity at pH 11.

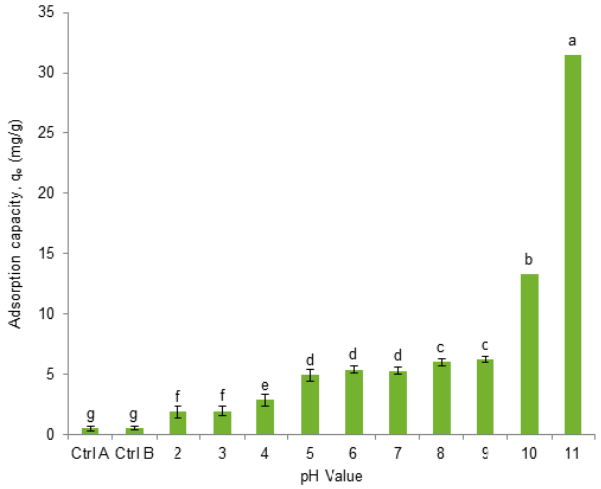



**Figure 6** Effect of pH of quercetin on the adsorption by weakly base ion exchange resin Amberlite IRA 67 (Volume: 20 ml; resin mass: 100 mg; temperature: 303 K; agitation speed: 120 rpm). A: Quercetin solution without resin and agitation, B: Quercetin solution without resin with agitation

The trend of these three phenolic compounds on adsorption capacity were quite similar. The adsorption capacity was low at highly acidic medium and gradually increased with increasing pH values up to pH 11. This might be due to the decrease in competition between the charged ions for the same functional group of the resin used. According to [28], a change of the $H_3O^+$ concentration in the solution resulted changes in the ratio of protonated/ unprotonated polyphenolic compounds. Protonation of these polyphenolic compounds significantly changes their charges as well the affinity to negatively charged adsorption resin Amberlite IRA 67. However, the results might be different from other phenolic compounds as phenolic profile vary in their molecular sizes and form which influences their solubility and adsorption [29].

### 3.2 Effect of Single Compound on Antioxidant Activity and Total Phenolic Compounds

Figure 7 indicates total phenolic content of the rutin solution at different pH ranges during adsorption process using Amberlite IRA 67. Sample control A and B did not give significant effect on agitation rate and time as discussed earlier. Sample controls of rutin solution significantly ($p<0.05$) showed the highest phenolic content compared to all rutin solution with pH modification. However, no significant different observed for rutin solution at pH 5 to other pH values except for pH 11. Rutin solution at pH 5 was only significantly different ($p<0.05$) to pH of 11. Overall, the trend shows pH did not give a significant effect on rutin solution in single compounds studied except at pH 11.

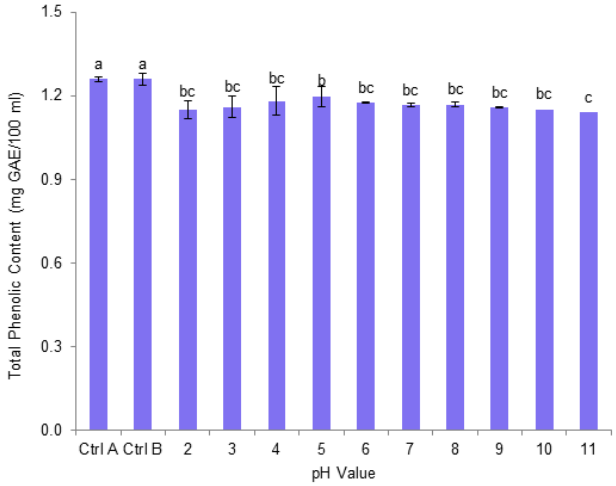



**Figure 7** The effect of TPC of single compound (rutin = 25 ppm) using Amberlite IRA 67 resin at different pH (Resin amount 100 mg; agitation rate: 120 rpm). A: Rutin solution without resin and agitation, B: Rutin solution without resin with agitation

The effect of ferric reducing activity (µmol/ g) of rutin solution on Amberlite IRA 67 were shown in Figure 8. From the figure, all rutin solution treated with the resin were significantly ($p<0.05$) lower compared to both control samples (A and B) for FRAP. There was no significant difference for FRAP between sample control A and B. Similar to adsorption capacity, agitation rate and time did give much effect on antioxidant losses. As can be seen in Figure 8, rutin solution at pH 5 shows the best ferric reducing activity with significantly ($p<0.05$) different to the rutin solution at pH 2, 3, 9, 10 and 11. The trend of ferric reducing activity of rutin solution might be due to the degradation rate constant with pH indicated that rutin solution was stable at lower acidic condition and lower alkaline condition [30].

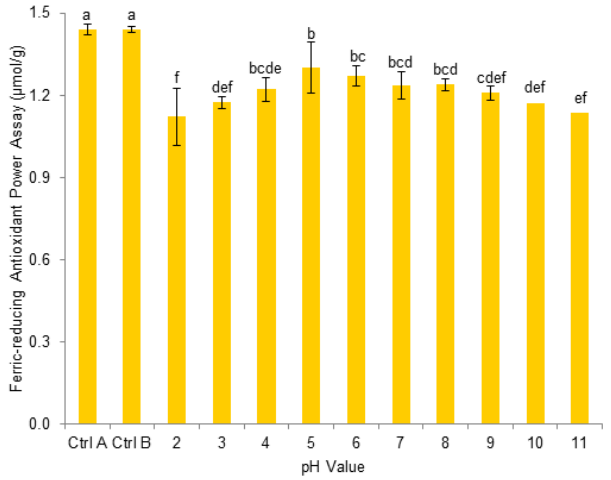



**Figure 8** The effect of antioxidant activity (FRAP) of single compound (rutin = 25 ppm) using Amberlite IRA 67 resin at different pH (Resin amount 100 mg; agitation rate: 120 rpm). A: Rutin solution without resin and agitation, B: Rutin solution without resin with agitation

Results of the free radical scavenging activity for rutin solution are presented in Figure 9. As expected, both control samples (A and B) are not significantly different but significantly ($p<0.05$) showed the highest among all rutin solution with pH adjustment. DPPH radical scavenging activity of rutin solution at pH of 2, 8, 9, 10 and 11 are considered significantly ($p<0.05$) lower as compared to the pH 3 to 7. There was no significant different at this pH range. As reported by [31], most phenolic compounds in plants show maximum polyphenoloxidase (PPO) activity at or near neutral pH values. The trend is similar with ferric reducing power of rutin where it shown the stability of the compound closer to the neutral pH regime. However, as the pH value increase, percentage of free radical scavenging activity gradually decreased. The lowest ($p<0.05$) scavenging activity of rutin solution was found at pH 11. Phenolic compounds are known as powerful chain breaking antioxidants [32]. Phenolic compounds including rutin are very important constituents of plants and their radical scavenging ability is due to their hydroxyl groups [30]. The phenolic compounds may contribute directly to antioxidative action [33].

Effect of pH during adsorption process of phenolic compounds has been investigated previously by many researchers. Most of them relate it to the degree of ionization of the phenolic sorbate. At pH > pKa the compounds are mainly in the form of negatively charged phenoxy ions, while the functional groups of the carbon surface are deprotonated and negatively charged. In addition to that, the electrostatic repulsion leads to a decrease of the adsorption capacity. On the contrary, when pH < pKa the phenolic compounds are predominantly in the neutral molecular form, while the surface acidity increases favoring the donor–acceptor interaction between the electrons of the aromatic ring and the surface. It leads to an increase of the adsorption capacity. Conversely, at low pH, the phenolic acids are hardly soluble in water. It means that for purification purpose only a narrow range of pH is possible: within this range the phenolic species must be water-soluble, i.e., slightly ionized, but not too much to be adsorbable onto the solid [34].

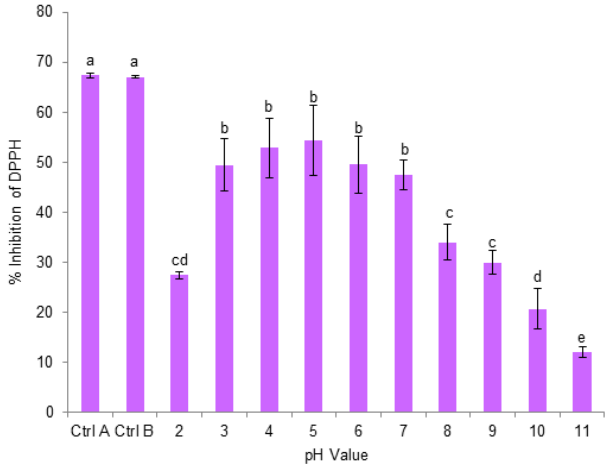



**Figure 9** The effect of antioxidant activity (DPPH) of single compound (rutin = 25 ppm) using Amberlite IRA 67 resin at different pH (Resin amount 100 mg; agitation rate: 120 rpm). A: Rutin solution without resin and agitation, B: Rutin solution without resin with agitation

Figure 10 shows total phenolic content of scopoletin solution at different pH range (pH 2 11). It was observed that no significant difference existed in both control samples (A and B) as observed in rutin solution. However, control samples significantly ($p < 0.05$) affected other scopoletin solution which undergo pH modification. Scopoletin solution at pH of 3 to 5 were not significantly different with the solution at pH 2, 6, 7 and 8 but decreased significantly ($p<0.05$) when it reached pH 9 and subsequently pH 10. Results showed significantly ($p < 0.05$) the lowest phenolic content of scopoletin solution at pH 11.

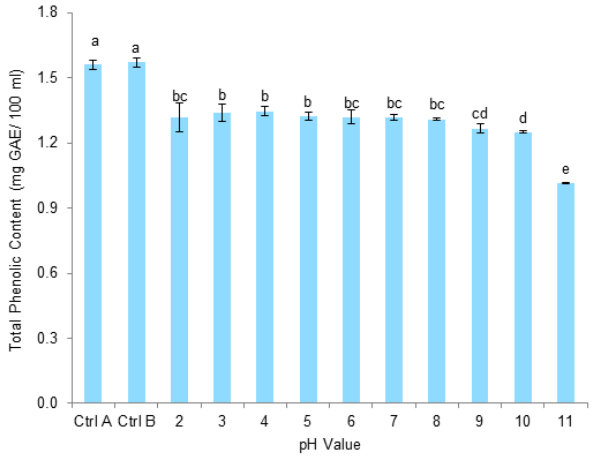



**Figure 10** The effect of TPC of single compound (scopoletin = 25 ppm) using Amberlite IRA 67 resin at different pH (Resin amount 100 mg; agitation rate: 120 rpm). A: Scopoletin solution without resin and agitation, B:Scopoletin solution without resin with agitation

According to Figure 11, the trend of ferric reducing power looks similar to total phenolic content of scopoletin solution. As expected, no significant difference was found between control samples (A and B) but the control samples showed the highest ferric reducing power. As for treated scopoletin solution, the solution adjusted at pH 4 significantly ($p<0.05$) showed the highest ferric reducing power as compared to other pH studied except for pH 3 and 5. The same phenomenon happened in rutin solution where the highest ferric reducing power or pH stability of the scopoletin was found at lower acidic condition.

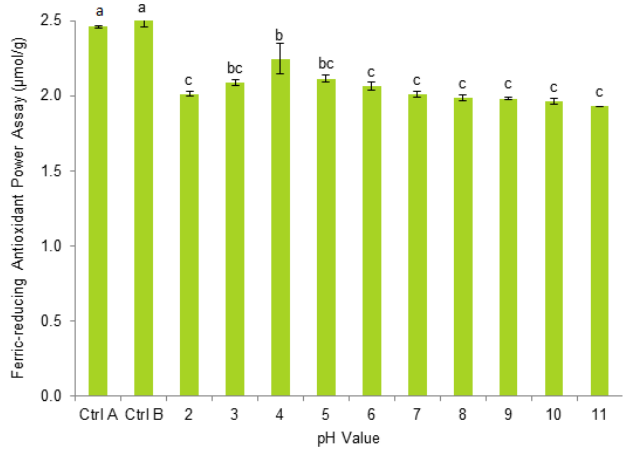



**Figure 11** The effect of antioxidant activity (FRAP) of single compound (scopoletin = 25 ppm) using Amberlite IRA 67 resin at different pH (Resin amount 100 mg; agitation rate: 120 rpm). A: Scopoletin solution without resin and agitation, B:Scopoletin solution without resin with agitation

The DPPH free radical scavenging activity of scopoletin solution at different pH ranges is shown in Figure 12. All treated samples of scopoletin solution using Amberlite IRA 67 resulted in significantly lower ($p<0.05$) free radical scavenging activity when compared to the control (A and B). At higher acidity (pH 2) of the solution, the scavenging activity seems slower and started to increase with increasing pH values up to pH 6. Scopoletin solution at pH 6 showed significantly ($p<0.05$) the highest free radical scavenging activity of the solution compared to the other treated solution. It shows DPPH free radical scavenging activity of scopoletin solution also was stability at lower acidic condition. Subsequently, scopoletin solution started to decrease gradually until it reached pH 11.

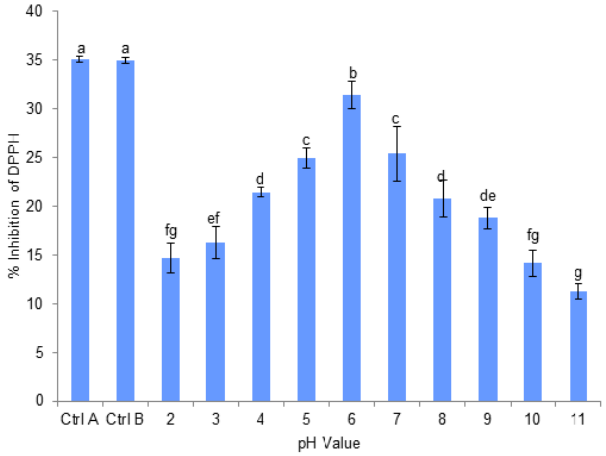



**Figure 12** The effect of antioxidant activity (DPPH) of single compound (scopoletin = 25 ppm) using Amberlite IRA 67 resin at different pH (Resin amount 100 mg; agitation rate: 120 rpm). A: Scopoletin solution without resin and agitation, B:Scopoletin solution without resin with agitation

Figure 13 shows total phenolic content of quercetin solution at different pH range (pH 2 to 11) during adsorption process of Amberlite IRA 67. As displayed, it was found that no significant difference existed between control samples A and B, whereas both samples have significantly ($p<0.05$) higher phenolic content compared to other treated samples with pH modification. However, for treated quercetin solution, the solution adjusted at pH 3 and 4 showed significantly ($p<0.05$) higher phenolic content compared to other samples except for pH 2, 5 and 6.

Figure 14 shows the effect of ferric reducing power for quercetin solution after treatment with Amberlite IRA 67 at different pH. No significant difference were found in control samples A and B but both samples were significantly ($p<0.05$) different compared to other treated quercetin solution. At pH 3 and 4, it showed the highest ($p<0.05$) ferric reducing power of treated quercetin solution except for pH 5. As the value of pH increase, the ferric reducing abilities of quercetin solution started to decrease. The pH value of the solution remains the same at pH 9 to 11 with the lowest ($p<0.05$) ferric reducing power.

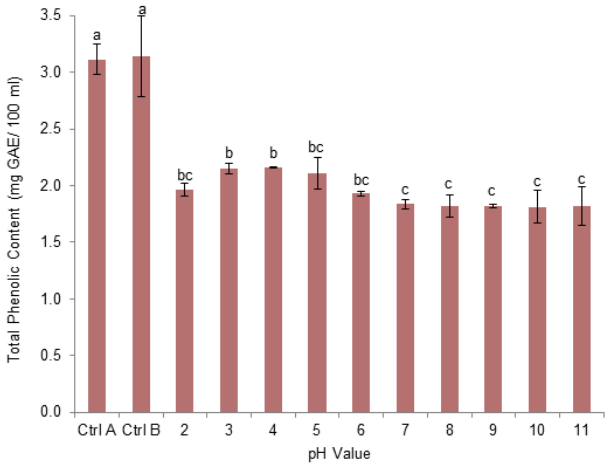



**Figure 13** The effect of TPC of single compound (quercetin = 25 ppm) using Amberlite IRA 67 resin at different pH (Resin amount 100 mg; agitation rate: 120 rpm). A: Quercetin solution without resin and agitation, B:Quercetin solution without resin with agitation

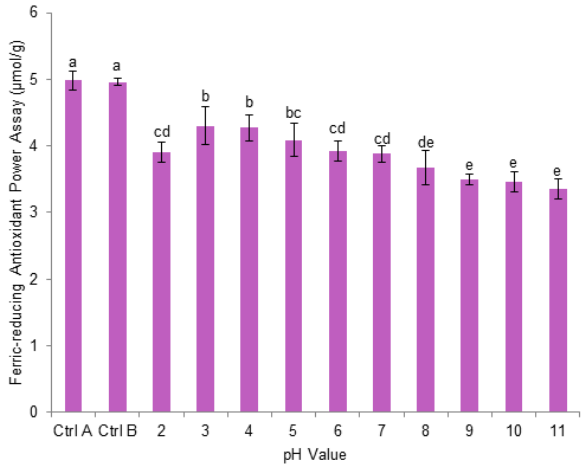



**Figure 14** The effect of antioxidant activity (FRAP) of single compound (quercetin = 25 ppm) using Amberlite IRA 67 resin at different pH (Resin amount 100 mg; agitation rate: 120 rpm). A: Quercetin solution without resin and agitation, B:Quercetin solution without resin with agitation

As can be seen in Figure 15, DPPH free radical scavenging activity of quercetin solution showed the highest percentage in control samples A and B. At high acidity (pH 2), the percentage of scavenging activity of treated quercetin solution was low. It started to increase significantly ($p<0.05$) at pH 3 and 4. When the pH was increased to pH 5 quercetin solution significantly ($p<0.05$) produced the highest scavenging activity compared to other treated quercetin solution. These results were similar to those obtained from the single compound of rutin and scopoletin solution where it shows the stability of the compound closer to the neutral pH regime. Beyond neutral pH, scavenging activity of quercetin solution gradually decreased as it reaches stronger base.

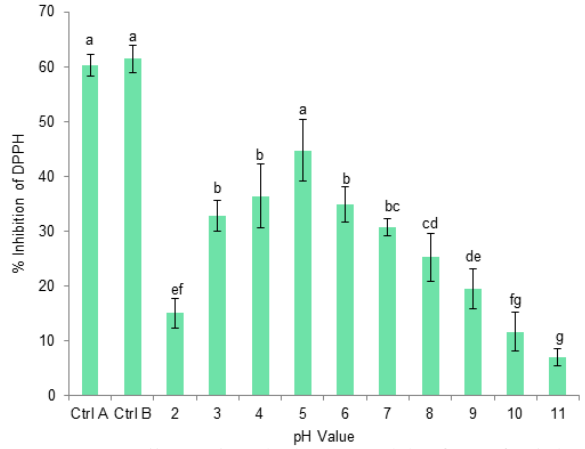



**Figure 15** The effect of antioxidant activity (DPPH) of single compound (quercetin = 25 ppm) using Amberlite IRA 67 resin at different pH (Resin amount 100 mg; agitation rate: 120 rpm). A: Quercetin solution without resin and agitation, B:Quercetin solution without resin with agitation

## 3.3 Effect of Multicompound on Adsorption of Organic Acids

In this model system studied, multicompound solution were used to stimulate actual noni juice using identical values of pH. As can be seen in Figure 16, there was no significant difference detected in both control samples A and B for hexanoic and octanoic acid. If compared to the adsorption capacity obtained in single solution, the trend observed for both compounds were similar. It shows an increase in adsorption capacity as the pH values increased. The highest adsorption capacity for hexanoic and octanoic acid were significantly ($p<0.05$) obtained at pH 10 to 11 and pH 9 to 11, respectively.

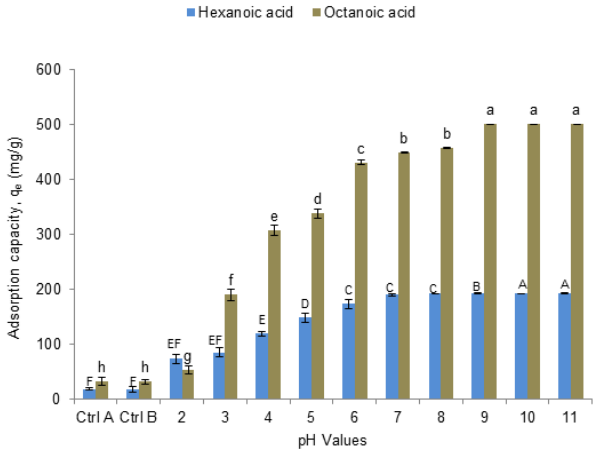



**Figure 16** The effect of organic acids of multicompound (A0 = 25 ppm; OA = 250 ppm; HA = 100 ppm) using Amberlite IRA 67 resin at different pH (Resin amount 100 mg; agitation rate: 120 rpm). A:Multicompound solution without resin and agitation, B:Multicompound solution without resin with agitation

*A-F Different capital letters in the same organic acid indicate significant difference ($p<0.05$)

*a-h Different letters in the same organic acid indicate significant difference ($p<0.05$)

### 3.4 Effect of Multicompound on Antioxidant Activity and Total Phenolic Content

The antioxidant activity and total phenolic content of multicompound in model system were also investigated at different pH values. Total phenolic content of multicompound solution during adsorption of Amberlite IRA 67 at different pH values is presented in Figure 17. The trend of the phenolic content was quite similar to those in single compound. As expected, no significant difference was found in control samples A and B. It means that agitation time and rate also did not affect both control samples (A, without resin and agitation and B, without resin). Changes in various pH values influenced the phenolic content in treated samples. A significant ($p<0.05$) reduction of phenolic content was observed for all samples treated with Amberlite IRA 67. No significant difference in multicompound solution between pH range 2 to 5 was observed. However, a decrease of phenolic content was found at higher pH values. The lowest phenolic content of multicompounds solution was obtained at pH of 11.

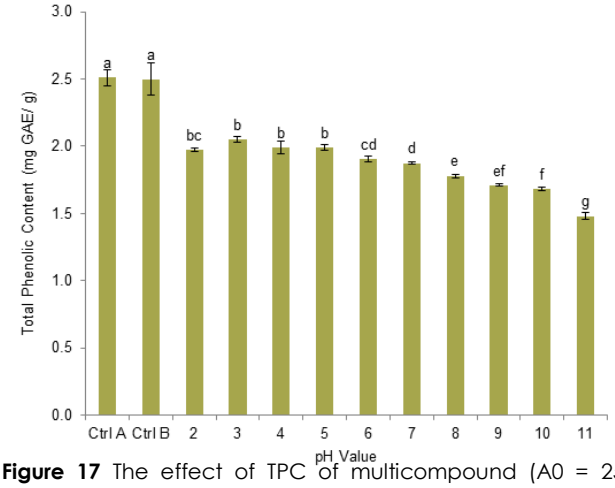



**Figure 17** The effect of TPC of multicompound (A0 = 25 ppm; OA = 250 ppm; HA = 100 ppm) using Amberlite IRA 67 resin at different pH (Resin amount 100 mg; agitation rate: 120 rpm). A:Multicompound solution without resin and agitation, B:Multicompound solution without resin with agitation

From Figure 18, it shows ferric reducing power of multicompounds solution during adsorption process using Amberlite IRA 67 at different pH. Similar to the trend in total phenolic content of multicompounds solution, no significant difference was observed between both control samples A and B. However, the ferric reducing power of multicompounds solution decreased significantly ($p<0.05$) in all multicompound solution treated samples with pH modification. At higher acidity (pH 2), the ferric reducing power was significantly ($p<0.05$) lower as compared to the multicompound solution at lower acidity (pH 3 to 6). The multicompound solution at pH 6 shows the highest ferric reducing power indicating the strongest ability to reduce Fe (III) to Fe (II). Furthermore, an increase in pH values decreased the ferric reducing power. The lowest ferric reducing power of multicompound solution was found at pH 10 and 11.

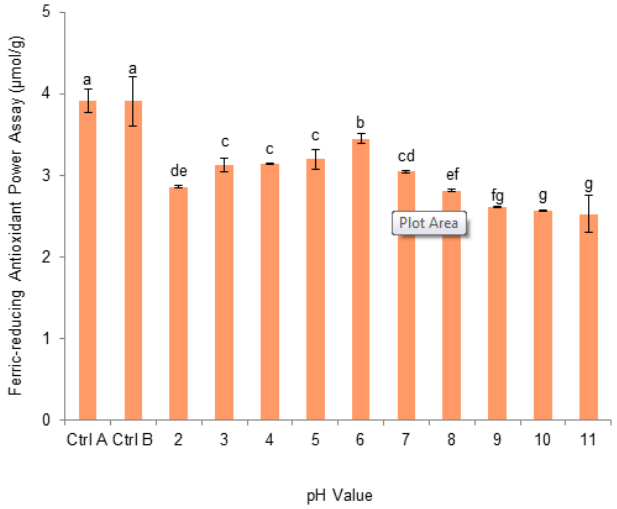



**Figure 18** The effect of antioxidant activity (FRAP) of multicompound (A0 = 25 ppm; OA = 250 ppm; HA = 100 ppm) using Amberlite IRA 67 resin at different pH (Resin amount 100 mg; agitation rate: 120 rpm). A:Multicompound solution without resin and agitation, B:Multicompound solution without resin with agitation

Figure 19 shows DPPH free radical scavenging activity of multicompound solution during adsorption process of Amberlite IRA 67 at different pH range. No significant difference was observed in control samples A and B. The DPPH free radical scavenging activity This observation was also not significant for multicompound solutions that were adjusted to pH 4 and 5. At higher acidity (pH 2), the scavenging activity of the solution seemed significantly ($p<0.05$) lower compared to lower acidity (pH 4 and 5) except for pH 6. The scavenging activity of the multicompound solution tremendously decreased as the pH increased. The lowest pH value of multicompounds solution obtained at pH 10 and 11.

There was a similar trend observed in antioxidant activity (FRAP and DPPH) of multicompound solution compared to those obtained in single solution. As mentioned by previous researchers [35], pH of the surrounding medium influenced the radical scavenging capacity of phenolic compounds. pH dependence for antioxidation activity against lipid oxidation and stable radicals can, at least in part, be related to the deprotonation of an OH moiety. The actual mechanism of antioxidant action of the deprotonated forms can be either hydrogen atom or electron donation or both [36].

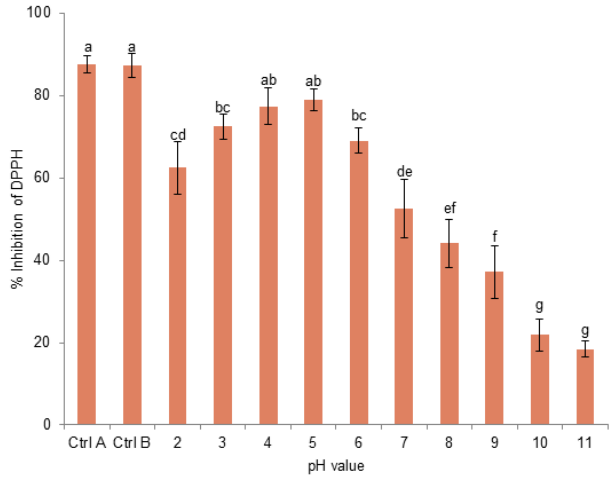



**Figure 19** The effect of antioxidant activity (DPPH) of multicompound (A0 = 25 ppm; OA = 250 ppm; HA = 100 ppm) using Amberlite IRA 67 resin at different pH (Resin amount 100 mg; agitation rate: 120 rpm). A:Multicompound solution without resin and agitation, B:Multicompound solution without resin with agitation

Figure 20 presents selected phenolic compounds (scopoletin, rutin and quercetin) in multicompound system at different pH range (2 to 11) including control samples. From the observation, there was no siginificant difference between both control samples (A and B) for scopoletin and quercetin but significantly ($p<0.05$) lower in treated samples with pH modification. There was no significant difference found in rutin control samples (A and B) and also rutin solution at pH 2 - 4. As the pH increased, each phenolic compound seemed to decrease until pH 11. The lowest concentration of each phenolic compound was determined at pH 11 which were significantly different ($p<0.05$) among other treated samples.

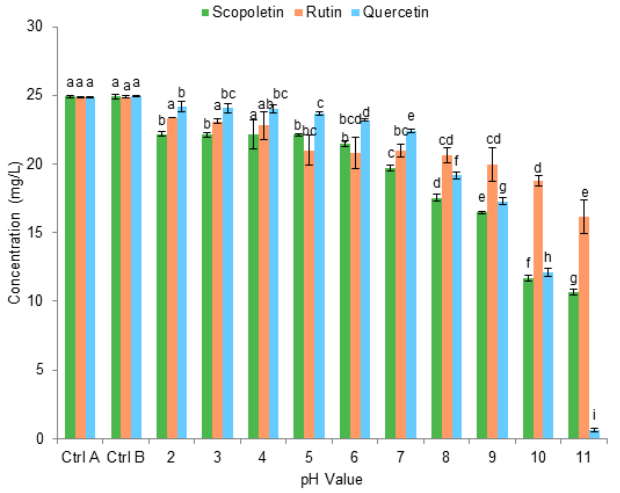



**Figure 20** HPLC determination of multicompound (A0 = 25 ppm; OA = 250 ppm; HA = 100 ppm) using Amberlite IRA 67 resin at different pH (Resin amount 100 mg; agitation rate: 120 rpm). A:Multicompound solution without resin and agitation, B:Multicompound solution without resin with agitation

*Different letters in the same phenolic compounds indicate significant difference ($p<0.05$)

The relationship between antioxidant compounds and antioxidant activities are complicated [37]. As mentioned by [38], antioxidant activity depends on the amount of antioxidants, structure and interactions among each other. The structure of phenolic compounds are an important determinant of the radical scavenging activity. According to [39], antioxidant activity rely on the number and position of the hydroxyl group in relation to the carboxyl functional group. In this case, it is also related to ionization process that occurs during pH modification of the samples studied. Therefore, high phenolic or flavonoid contents do not necessarily exhibit high antioxidant capacity. These results agree with [40, 41] and [37] who reported that differences in antioxidant activities could be due to the different qualitative compositions of phenolic constituents.

## 4.0 CONCLUSION

It was shown that the pH dependence of adsorption capacity of weak base anion exchange Amberlite IRA 67 resin had similar increasing trend on organic acids (hexanoic and octanoic acid) and phenolic compounds (rutin, scopoletin and quercetin) studied as pH values increased. In single solution of each phenolic compound, it was observed that total phenolic content (TPC) and antioxidant activity (FRAP and DPPH) gave highest values closer to neutral pH regime. The pH dependence of adsorption capacity in multicompounds solution also showed similar trend for organic acid compounds. Similar trend was also found in multicompounds solution of phenolic compounds in total phenolic content (TPC) and antioxidant activity (FRAP and DPPH). The findings of the present study are important for further investigation to be applied in actual system.

## Acknowledgement

The authors would like to thank The Ministry of Education (MOE) for the scholarship, The Ministry of Science, Technology and Innovation (MOSTI) for financing the project under the ERGS/1/2013/STWN03/UKM/02/1 grant and Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia.

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