

New Innovative Valve for Flow Injection to Determine the Total Phenols and Antioxidant in Mushroom

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Abstract

In this study a new valve B had been designed and constructed in the analytical laboratory by using local available facilities and resources. This was applied to determine the total phenols (TP) in plants extracts using gallic acid (GA) as a standard. The results were compared to those obtained from a standard valve A. It was found that under the optimized experimental conditions and using calibration graphs with range of 1.0 - 40.0 mgL⁻¹ ($r^2=0.9986$) and 1.0–50.0 mgL⁻¹ ($r^2=0.9993$) for the valves A and B respectively, the limit of detection (LOD) in the aqueous solution was 0.05 mg.L⁻¹ for both valves. Total phenol content and antioxidant activity of methanol extracts from mushroom were determined and compared to those values obtained for gallic acid. Flow injection analysis (FIA) determination of the TP content was performed using the Folin-Ciocalteu Reagent (FCR), while antioxidant activity was measured by reaction with 1,1-diphenyl-2-picryl RYdrazyl radical (DPPH assay). The values of phenolic compounds content and antioxidants activity which were determined by valve B were notably higher than these in valve A. Finally, both valves A and B showed high sensitivity, flexibility, extend range of linearity, rapidity and ease of use. Also their material were not expensive, therefore using them is successful to determine the total phenols and antioxidants.

Key words: FIA, Folin-Ciocalteu Reagent, DPPH assay, mushroom, total phenols

الخلاصة

تم تصميم وتركيب صمام جديد (ب) لمنظومة الحقن الجرياني في مختبر الكيمياء التحليلية باستعمال مواد متوفرة تجارياً، وقد استعمل هذا الصمام في تقدير الفينولات الكلية ومضادات الاكسدة في المستخلصات النباتية باستخدام حامض الكاليك كمحلول قياسي. تم مقارنة النتائج مع تلك المستحصلة من استعمال الصمام (أ) وتحت نفس الظروف التجريبية للصمامين حيث وجدت الخطية ضمن مدى (1.0-40.0 mgL⁻¹) ($R^2 = 0.9986$) و (1.0-50.0 mgL⁻¹) ($R^2 = 0.9993$) للصمامين (أ) و (ب) على التوالي، مع حد كشف (LOD = 0.05 mgL⁻¹) لكلا الصمامين. المحتوى الكلي للفينولات وفعالية مضادات الاكسدة للمستخلص الكحولي للفطر قدرت وتمت المقارنة بين النتائج المستحصلة لحامض الكاليك حيث تم تقديرها باستعمال تقنية الحقن الجرياني واستعمال كاشف فولن للكشف عن الفينولات الكلية بينما استعمل كاشف (DPPH) لقياس فعالية الاكسدة وقد لوحظ ان قيم المركبات الفينولية ومضادات الاكسدة التي قيست باستعمال الصمام (ب) كانت نوعاً ما اعلى من تلك المقاسة باستعمال الصمام (أ). في النهاية كلا الصمامين اظهرا مرونة وحساسية عالية، مدى واسع من الخطية، سهولة في الاستعمال، سرعة في القياس كذلك فان المواد الاولية المستعملة في تركيب الصمامين متوفرة ورخيصة الثمن ولهذا تم استعمالها بنجاح في تقدير الفينولات الكلية ومضادات الاكسدة.

الكلمات المفتاحية: الفينولات الكلية، مضادات الاكسدة، تحليل الحقن الجرياني، تصميم صمام حقن جرياني، كاشف فولن

1. Introduction

Mushrooms have been used widely, in many countries, as a nutritious food because of their high content of vitamin B, fiber, minerals, and amino acids, beside their low fat content. Moreover, mushrooms considered as great medical raw materials because of their potential ability to minimize the risk of cancer, reduce cholesterol level, and prevent different many diseases like hypertension. In addition, they contain many types of antioxidants such as flavonoids and polyphenols (Mujic *et al.*, 2010; Parrilla *et al.*, 2007). Many epidemiological studies proved that high polyphenol content foods and beverages, which exhibit antioxidant activity, play an essential role in the enhancing of human immunity and reduce the risk of stroke, certain types of cancer, and cardiovascular diseases (Nuran *et al.*, 2012). As well as, it has been found that the antioxidants inhibit the oxidative stress in our bodies as they have the ability to slow the oxidation rate through scavenging of free radicals (Luis *et al.*, 2009, Gan *et al.*, 2013). Moreover, mushrooms represent an unexploited source of effective pharmaceutical products (Hima *et al.*, 2012, Anita *et al.*, 2009) and they contain a high concentration of phenolic compounds (Shirmila *et al.*, 2012, Lillian *et al.*, 2007).

The ability of mushrooms to scavenge free radicals, like lipid peroxy and peroxide, results in preventing the oxidative mechanisms and enhancing anti-carcinogenic and degenerative for a wide range of diseases (Hip *et al.*, 2009). *Fomes fomentarius*, *Trametes versicolor* and *Ganoderma applanatum* are the three main wild edible mushrooms that contains high concentration of total flavonoid, total phenolic, and condensed tannins (Abugria & McElhenney 2013).

In the present paper the improved FIA-FC, FIA-DPPH^{•+} methods were useful to screen rapidly, without dilution, and with high repeatability. The same flow injection system was later applied to determine the total phenols and antioxidant capacity of mushroom using standard gallic acid. The FC method were applied for the determination of the total phenols contents, and DPPH method for the determination of antioxidant activity of phenolic compounds using two valves as a unit of FIA, one of them was new homemade designed.

The aim of the research is to apply the basic facts about suitability of flow injection analysis techniques and their combination with folin-ciocaltue reagent for assay of phenolics in mushroom, and to examine the ability of the new homemade valve to determine the phenolic content in mushroom, including the different parameters that affect the determination of phenolic compounds, such as length of loops, temperature, length of reaction coil, concentration of reagent, dispersion. The sensitivity, selectivity and reproducibility in the case of quantifying phenolic compounds of the FIA techniques with spectrophotometric detection systems make them the best method to analysis of phenolic compounds (Cortina-Puig 2012; Merken & Beeche, 2000).

2. Chemicals and Samples

All chemicals and reagents were of analytical grade and used without further purification. Folin Ciocalteu (EC)-No 127212008), DPPH free radical (2,2-Di(4-tert-octylphenyl)-1-picrylhydrazyl) (CAS-No 84077-81-6), gallic acids (CAS-No 149-91-7), ascorbic acid (CAS-No 50-81-7), sodium carbonate (CAS-No 497-19-8), potassium ferricyanide (CAS-No 13746-66-2), trichloro acetic acid (TCA) (CAS-No 76-03-9), ferric chloride (CAS-No 7705-08-0), disodium hydrogen phosphate (CAS-No 7558-79-4) and sodium dihydrogen phosphate (CAS-No 7558-80-7) were purchased from Sigma–Aldrich (Germany). The polyphenol stock solutions were prepared by dissolving an appropriate amount of the compound in 80% methanol (Environmental grade, Alfa Aesar, Barcelona, Spain). All the stock solutions were kept away from the light and stored for not more than 48 hours. Diluted solutions were prepared daily from these stock solutions. Mushroom samples were purchased from different local markets.

3. Instruments and Apparatus

Flow injection analysis set up consisted of; a peristaltic pump (model ISM 834; Ismatec, Germany). This was connected to low-pressure homemade valve which contains ten ports with three loops. Centrifuge (EBA 20 Hettich, 5000 rpm, Germany), shaker water bath (Memmert, Germany), balance (Denver instrument, Germany), Heater (ardeas 51, Germany). Measurements were carried out using a double-beam spectrophotometer APEL PD-303UV detector with flow cell 394 μ L (Japan). Recorder (Siemens C 1032, Germany). UV-VIS spectrophotometer (shimadzo 1800, Japan). Valves were made in the laboratory from sub-valve (SV1,SV2,SV10), while the reaction coil was made in a glass workshop.

4. Experimental Design

An innovated homemade valve (Fig. 1) with a simple homemade valve that was designed and developed of FIA system by Dakhil N.Taha (Figs.5) (Taha 2003), were used for all experiments. Peristaltic pump (Ismatec, Germany) with bridged PVC tubing (1.02mm i.d.) propelled carrier streams at total flow rate of 2.0 mL min⁻¹. Analytes and standards were manually injected into a water carrier stream using a ten-port innovated homemade injection valve (plastic Instruments), which merged and passed through reaction coil to flow cell. The detection system consisted of a UV detector.

4.1. Selection of Two Loops (Valve B)

The antioxidant activities of standard and sample solutions were estimated according to FIA-DPPH method. For loading of two components (gallic acid and DPPH assay) into two loops, choosing loops (L₃, L₂) because they were close to each other and they had the same distance from the mixing point, which made the process more efficient. The process of loading DPPH solution on L₃ was carried out to close the sub-valves SV2 & SV7 towards b

and open towards (c-a), other sub-valves were opened towards (a-b) and closed towards the (c) Fig. (A), while the gallic acid was loading on L_2 with the same processes of loading L_3 Fig (B). The washing process of the remains of the components inside the cavity of main valve; all sub-valves closed towards (c) and opened towards (a-b), except SV5 was closed towards (a) and opened towards (c-b), and SV3 was opened at all directions (a-b-c) as a Fig.3. Injection process takes place after the close of SV2, SV7 towards (b), and open towards (a-c), and SV4, SV9 were closed toward (a) and opened toward (b-c) and SV3, SV8 opens at all directions (a-b-c). Then allow to the carrier stream to pass through the valve to introduce the components to flow. These processes were shown in Fig 1(C).

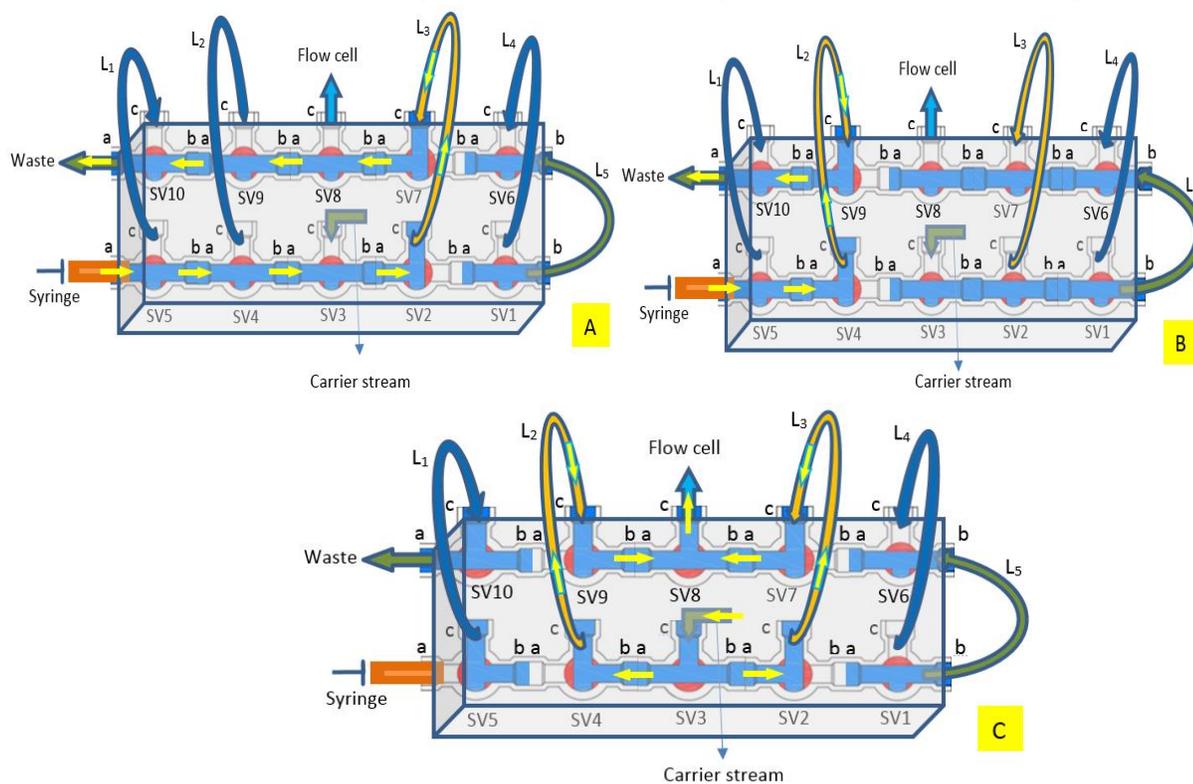


Fig. 1. Select two loops for inject the sample and reagent, A loading reagent on L_3 , B loading sample on L_2 and C was shown passing the carrier stream through the valve to introduce the components into the flow cell.

4.2. Selection Three Loops:

The processes for injection of three components to flow cell, were done to load three loops (L_4, L_3, L_2) with three components (gallic acid on L_2 , FCR on L_3 , buffer solution on L_3 when used FIA-FCR and $FeCl_3$ on L_2 , ascorbic acid on L_3 , P.B.T mixture on L_4 when used FIA-FRAP). To load a third component on L_4 , the sub-valve SV1, SV6 were closed towards (b) and opened them towards (a-c). All the others sub-valves were closed towards (c) and opened towards (a-b). In the same way the loading of L_3 with the second component was by closing of SV2, SV7 towards (b) and opening them towards (a-c), and loading L_2

with the first component by the close of SV4, SV9 towards (a) and opening them towards (b-c). The rest of the valves must be kept close towards (c) by using syringe to inject the components on loops, these processes were as shown in Fig. 2 (D,E). The washing process for the remaining of the components inside the cavity of the main valve. This process was conducted after loading of all components. All sub-valves must be close towards (c) and opened towards (a-b), except SV5 must be closed towards (a) and open towards (c-b), and keep SV3 open at three directions (a-b-c). The injection process took place after closing SV1, SV6 towards (b), and opening them towards (a-c). SV4, SV9 must be closed towards (a) and opened towards (b-c), SV3, SV8 were opened to three directions (a-b-c), and then the carrier stream was allowed to pass through the main valve to introduce the components into the flow cell, as shown in Fig. 3.

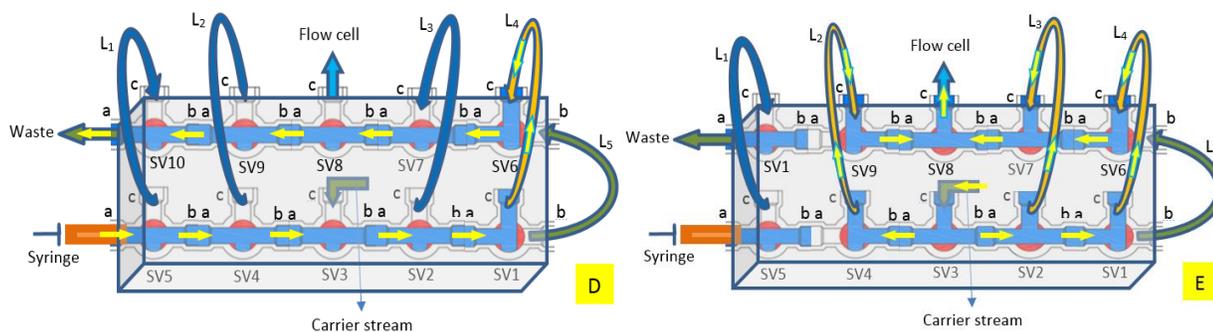


Fig. 2. Selection of three loops to inject the sample with two components, this process is done by loading L_4, L_3, L_2 , as shown in Fig. 1 (A,B) and Fig. 2 (D). The carrier stream was allowed to pass through the valve to introduce the components into the flow cell as shown in (E).

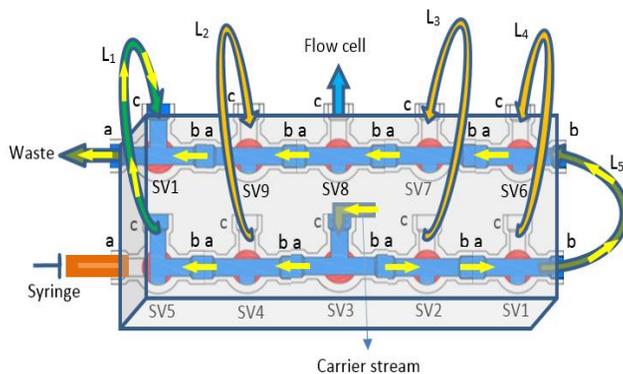


Fig. 3. Washing process was done after loading each component which represents with orange loops, the green loops represent the passage of the carrier stream through the valve to remove residual components inside the cavity of valve.

5. Optimization

The total phenols of standard and sample solutions were estimated by the FIA-FCR method was optimized with the physical and chemicals conditions for the FIA technique as follow; Gallic acid is loaded in L_2 (117 μL), buffer solution (7.5% Na_2CO_3) was loaded in L_4 (235 μL), the reagent (Folin-Ciocalteu) was loaded in L_3 (157 μL), length of reaction

coil was 100 cm, the carrier stream was distilled water. Flow rate $2 \text{ mL} \cdot \text{min}^{-1}$ was at the temperature of $30 \text{ }^\circ\text{C}$, the response measured at 760 nm. Determination frequency was 55 per hour. These data are shown in Table 1.

Table 1. Analytical values of statistical treatment with optimum working conditions for the GA determination with FCR system.

Parameters	Length of R.C	Flow Rate	Loading Site	volume of Loops	Temp	Buffer Soln.	FCR Conc.	Replication	LOD & LOQ
Optimum value	100 cm	$2 \text{ mL} \cdot \text{min}^{-1}$	GA(L ₂) FCR(L ₃) Buf. (L ₄)	(L ₄ =235 L ₃ =157 L ₂ =117) μL	$30 \text{ }^\circ\text{C}$	7.5% w/v	0.2M λ_{max} 760 nm	$\bar{d} = 5.02$, SD =0.124, RSD% =2.48	Math.=1.41 mgL^{-1} , Prac.=50 $\mu\text{g} \cdot \text{L}^{-1}$, LOQ = 4.28 mgL^{-1}

5.1. Calibration Graph for Innovative Valve B

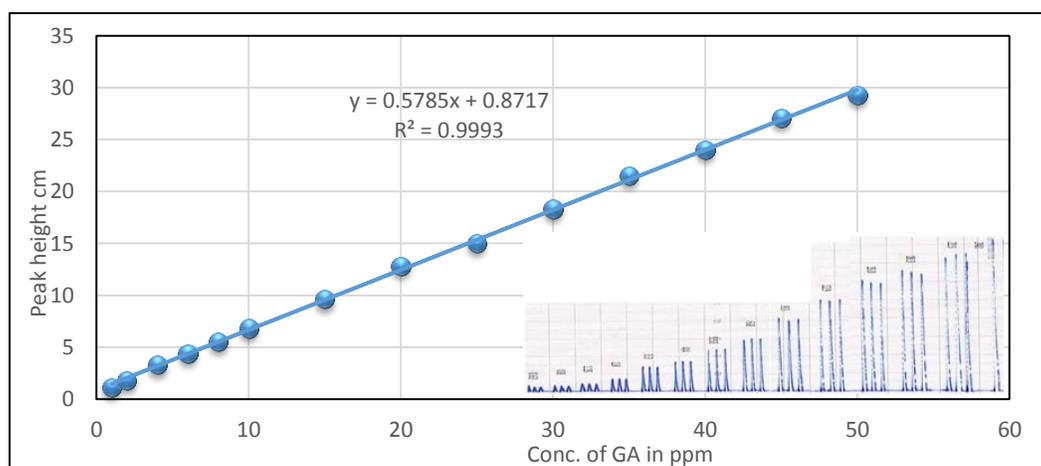


Fig. 4. Calibration curve of different concentration of gallic acid responses expressed as an average peak height cm, which reacts with 0.2M FCR. Valve B

5.2. Calibration Graph. (Valve A)

The calibration graph of GA concentration was studied over the range of (0.2–40) ppm of standard gallic acid and 0.2 M FCR which was prepared from stock solution (2M FCR). The response measurements were recorded under the optimum conditions. The A valve was set as shown in fig. 5. (235 μL) buffer solution (7.5% w/v Na_2CO_3) was loaded on L₃, (157 μL) FCR was loaded on L₂, (117 μL) GA solution was loaded on L₁, and distilled water was the carrier stream with flow rate ($2 \text{ mL} \cdot \text{min}^{-1}$). The processes were conducted as illustrated in Fig. 6. These processes showed an increasing response which expressed as an average peak height (N=3) with the increasing of the GA concentration.

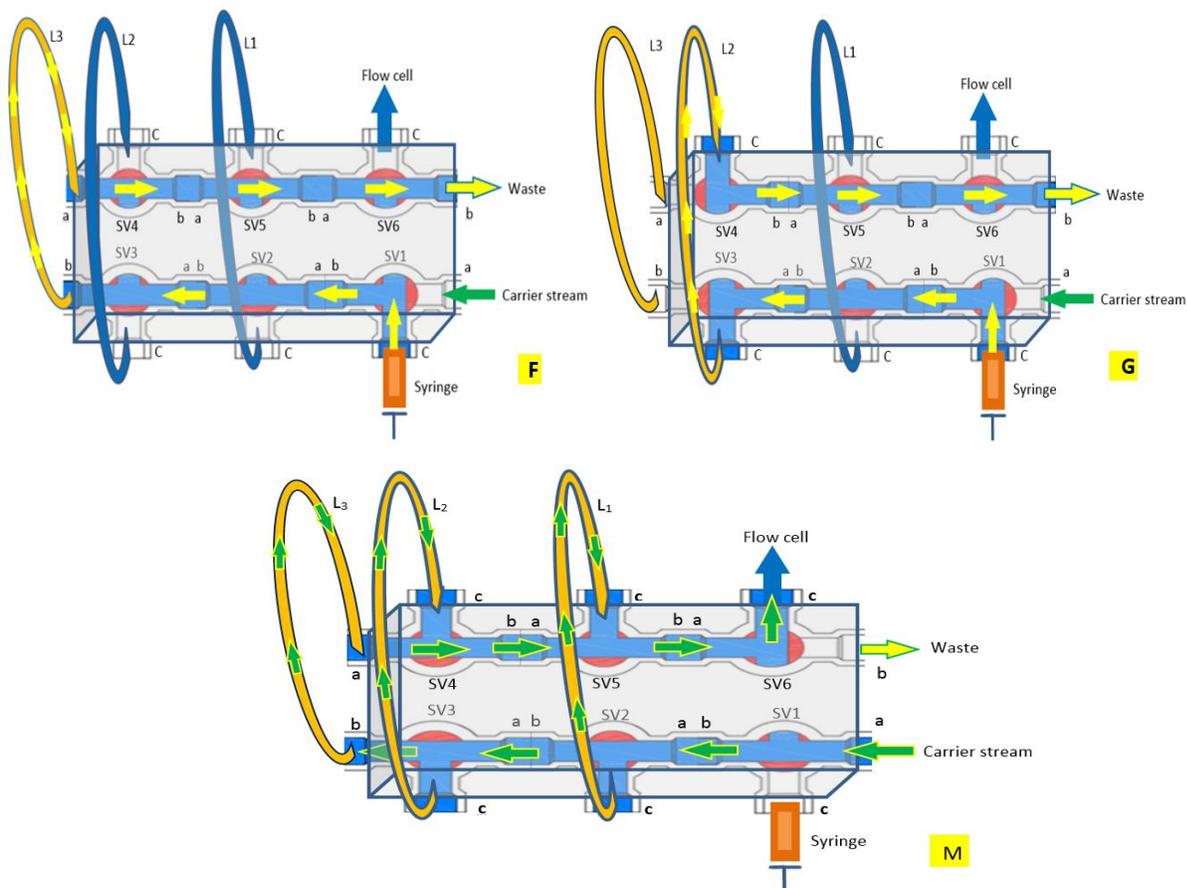


Fig. 5. The loading components on L₃, L₂, L₁ represented by F, G, H. While M showed the injection process of three loops components to flow cell, by moving all sub-valves towards three directions (a,b,c) with blue colour except sub-valves (SV1, SV6) were closed toward (c, b) and opened toward (a-b , c-a) with red colour.

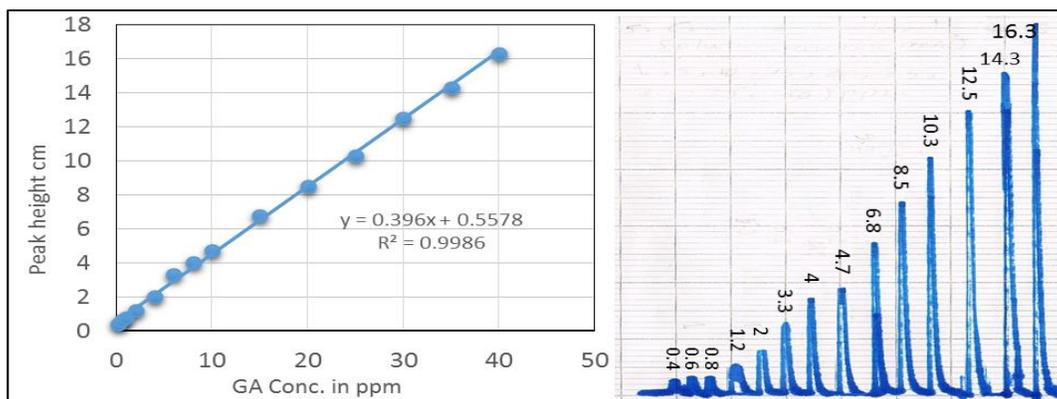


Fig. 6. Calibration graph of standard gallic acid expressed as an average height peak (cm) versus variation conc. of GA (ppm) which reacts with 0.2 M FCR, for (valve A).

5.3. Analytical Characteristics

In this study, the standard solution of gallic acid was used to prepare the calibration curve of the proposed FIA system under the optimum conditions, and examine the linear range, precision, accuracy, limit of detection (LOD) and sampling frequency. The calibration graph was obtained by plotting the response (N=3) of the solutions as a peak heights against the standard concentrations that gave linearity over the concentration range from 0.2 to 50 mgL⁻¹ of gallic acid for valve B and were expressed in the regression equation $y = 0.5785x + 0.8717$ with a correlation coefficient of $r^2 = 0.9993$ as shown in Fig. 4, while the range of linearity for valve A was from 0.2 to 40 mgL⁻¹ of gallic acid and was obtained with the regression equation $y = 0.396x + 0.5578$, correlation coefficient (r^2) = 0.9986, as shown in Fig. 6. Where y was peak height in cm and x was gallic acid concentration in mgL⁻¹. Practically the limit of detection (LOD) was found to be 50 µg.L⁻¹ for both valves. TP contents in the sample solutions were determined by using this calibration curve.

6. FIA-DPPH Method to Determine of Antioxidants.

New design valve of the FIA system was used for the determination of antioxidant activity. The change in colour that was caused by the reaction between free radical and sample was measured as a peak height. The sensitivity of the measurement depended on the choice of free radicals, reaction time, length and shape of reactor as well as flow rate (Giovana *et al.*, 2011). Choice of test substances and reagents for the FIA, GA and DPPH were used as test substances to measure the antioxidant activity of TP in the extracts, using FIA system optimization. The antioxidant activity could be measured from the decrease of the absorbance of a free radical (R•) after reacting with an antioxidant (AH) (Emad & Sanaa 2013):



6.1. Selection of Detection Wavelength.

DPPH as standard control solution was prepared according to the standard procedure. The maximum absorption wavelength of the purple colour product was 517 nm (Adeolu & Florence 2009).

6.2. Injection Process of DPPH and Determination of Free Radical-scavenging Activity (RSA%).

This process was determined by modification of the Blois method. Suitable volumes of 0.2 mM 80% methanolic solution of DPPH free radical was loaded on L₃, Suitable volumes of standard gallic acid solution or crude extract methanol solution of TP was loaded on L₂ (containing 50 – 400 µg of dried extract). The carrier stream (distilled water) was allowed to transfer the components to the flow cell passing through the reaction coil (Fig. 1). The response was measured at 517 nm. The activity was given as % DPPH radical scavenging calculated according to the following equation: % DPPH Radical Scavenging

Activity (RSA%) = [(control response (Ctrl) – extract response)/control response (Ctrl)] x100 (Sarini &Nor' Aishah 2014).

6.3. Effect of DPPH & GA Concentration.

After establishing the physical conditions of the system, the effect of DPPH and GA concentration on the response signal was also studied to ensure that their concentrations were not the limiting one in this application range. The concentration of DPPH was studied in the range of (0.1- 0.5) mM. For concentrations higher than 0.2 mM of DPPH give no significant increase on the response, therefore this concentration was chosen. GA concentration was studied in a range of (1 - 25) ppm. At concentrations higher than 25 ppm there was no significant increase in the response as (RSA%), and this is illustrate in Figures 7, 8. The optimization conditions were shown in Table 2.

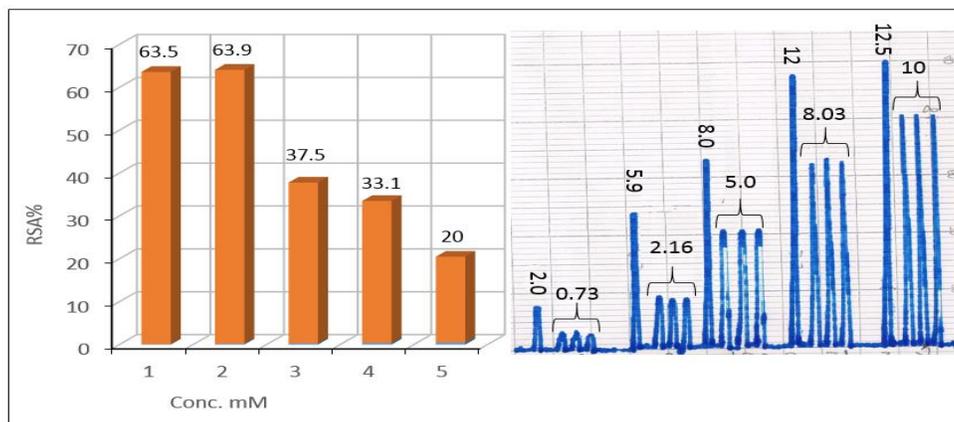


Fig. 7. Relationship between the RSA% expressed as average height peak (cm) versus deferent concentration of DPPH, using constant concentration of GA.

Table 2. Analytical values of statistical treatment with optimum working conditions for determination of RSA% (DPPH) with GA concentration.

Parameters	Length of RC	Flow Rate	Loading Site	Volume of Loops	Temp	DPPH Conc.	IC50	LOD
Optimum value	150 cm	2 mL. min ⁻¹	GA (L2) DPPH (L3)	L3,L2 (235µL)	30 °C	0.2 mM	IC ₅₀ = 0.105mg/mL for 0.2mM DPPH, 0.137mg/mL for 0.4mM DPPH	Practically 50µg.L ⁻¹ (GA) 0.1mM (DPPH)

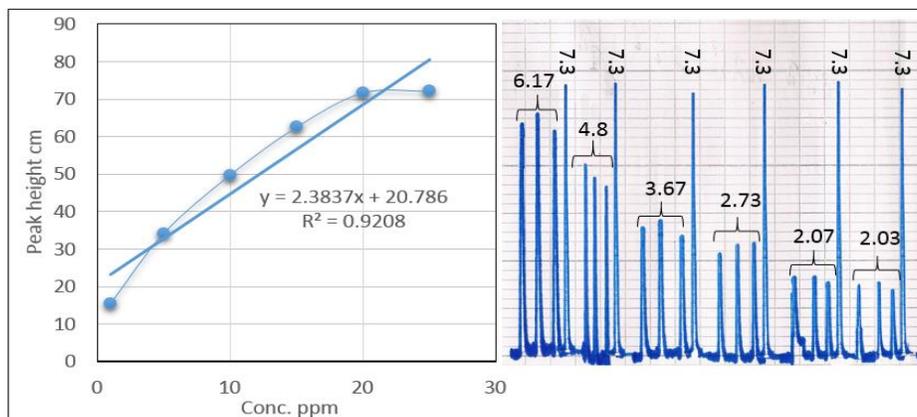


Fig. 8. The relationship between the RSA% expressed as an average peak height (cm), versus different concentration of GA, using 0.2 mM DPPH.

$IC_{50} = 0.105\text{mg/mL}$

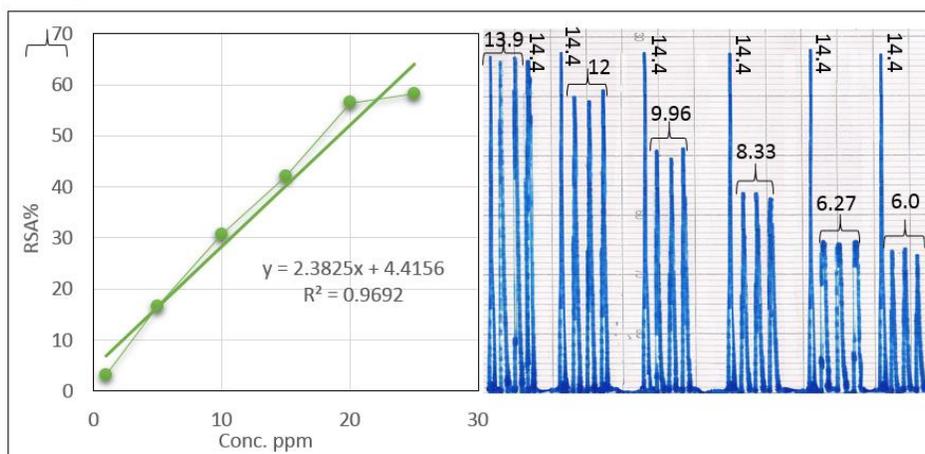


Fig. 9. The relationship between the RSA% expressed as average height peak (cm), versus different conc. of GA, using constant conc. of DPPH at 0.4 mM.

$IC_{50} = 0.137\text{mg/mL}$

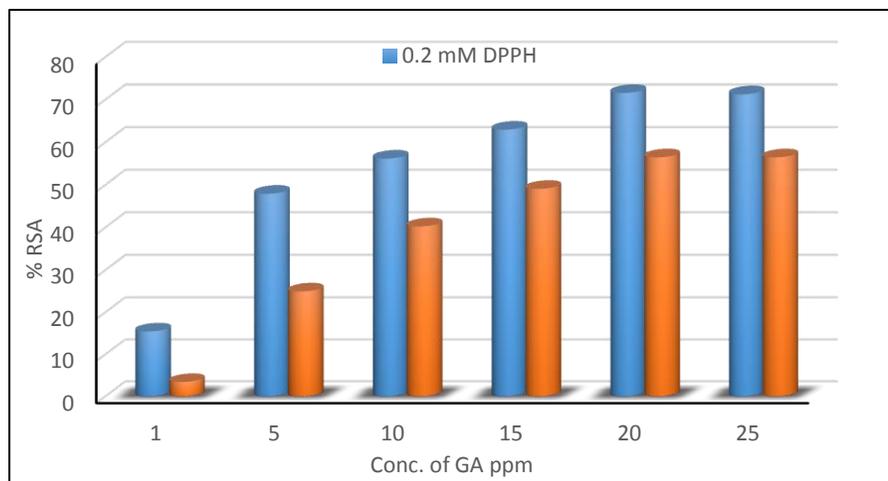


Fig. 10. The relationship between the RSA% expressed as average height peak (cm), versus two different concentration of DPPH reagent (0.2, 0.4) mM, using constant concentration of GA at 10 ppm.

7. Application of the Proposed Method to Mushroom Sample

7.1. Samples Preparation

The fruiting bodies of mushroom were cleaned and washed to remove any residual composition by using distilled water. These samples were cut into pieces and dried in shade at room temperature (26 °C), then dried at (40 °C) to constant weights. Using oven dryer to remove the moisture content. After drying, the dried samples were grinded into fine powder by using micro-grinder and kept in plastic bag prior to analysis (Rabeta & Faraniza.2013)

7.2. Extraction of Polyphenols

The method that was described by the International Organization for Standardization (ISO) 14502-1 was used. Briefly, 0.200 ± 0.002 g of grinded mushroom was weight in an extraction tube, and 10 mL of 80% methanol at 50 °C was add. The extract had mixed by using a vortex machine and shaken in a water-bath at 50 °C for 2 hr. After cooling at room temperature, the extract centrifuged at 5000 rpm for 10 min.; the supernatant decanted in a graduated tube. The extraction step repeated twice. Both extracts had pooled and the volume adjusted to 20 mL with cold 80% methanol. (1, 2, 3, 4, 5) milliliter of the extract was diluted with 80% methanol to 20 mL (Claudia 2008).

7.3. Determination of Total Phenolic Content and Antioxidant Activity

Phenolic compounds in the extracted sample were estimated by using modification of Folin-Ciocalteu assay, based on procedures described by Singleton and Rossi (1965) (Olusegun et al 2013, Vijayakumar &Kumar 2013). After optimization of the conditions for determination of phenolic content by the FCR spectrometry method which was described as

a peak height with minor modifications to FIA suitability. Mushroom extract (117 μL), FCR (157 μL) and buffer solution (235 μL) were injected into L_1, L_2, L_3 for valve A and L_2, L_3, L_4 for valve B. Response of samples was measured at 760 nm, flow rate of 2 $\text{mL}\cdot\text{min}^{-1}$ and each sample was analyzed in triplicate. Antioxidant activity and total phenol (TP) content of 80% methanol extracts of mushroom were determined and compared with the obtained values for gallic acid. FIA determination of the TP content had performed using the FIA-FCR method, while antioxidant activity had measured using FIA-DPPH method. The TP content had observed for mushrooms in comparison to gallic acid analyzed.

7.4. Determination TP and antioxidants of Mushroom. (Valve B)

7.4.1. FIA-FCR Determination

The volume of extracts had pooled and adjusted to 20 mL with cold 80% methanol. (1, 2, 3, 4, 5) mL of the extract was diluted with 80% methanol to 40 mL, then total phenols were determined using FIA-FCR method.

7.4.2. FIA-DPPH Determination

IC_{50} was determined from the linear regression equation. Regression equations had correlation coefficients ≥ 0.91 . The IC_{50} was expressed as μg solids/mL DPPH solution and the antioxidant activity of the sample was reported as $1/\text{IC}_{50}$, $\text{IC}_{50} = 80.68 \mu\text{g/mL}$ DPPH, $1/\text{IC}_{50} = 12.4 \text{ mg/mL}$ DPPH.

Table 3. Summary results for determination of total phenols in Mushroom using 0.2 M FCR.

Volume of MS E/M	Conc. in ppm	Peak height cm NO. of replicate (N)			Mean \bar{d}	SD	RSD%	TP mg/g GAE	$\bar{d} \pm t \frac{SD}{\sqrt{n}}$
		1	2	3					
1/40	3.27	2.8	2.7	2.6	2.7	0.08	3.02	13.0	1.86 ± 0.24
2/40	6.0	4.4	4.4	4.4	4.4	0.0	0.0	12.0	4.4 ± 0.0
3/40	9.3	6.4	6.3	6.3	6.33	0.04	0.74	12.8	6.33 ± 0.14
4/40	14.8	9.4	9.5	9.5	9.47	0.04	0.49	14.8	9.47 ± 0.14
5/40	19.0	12.6	12.2	11.8	12.2	0.32	2.67	15.2	12.2 ± 0.99

The average of total phenols in mushroom (MS) = $13.56 \pm 0.3 \text{ mg/g}$ GAE

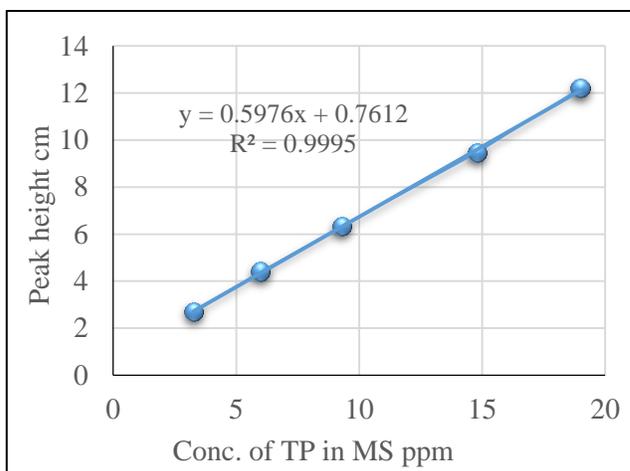


Fig. 11. The relationship between the responses expressed as an average peak height cm versus deferent concentrations of total phenols in mushroom using 0.2 M FCR.

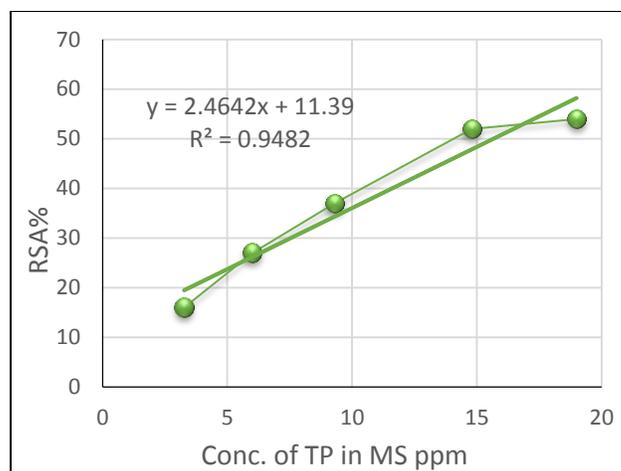


Fig. 12. The relationship between the RSA% expressed as an average peak height cm versus deferent concentrations of mushroom using 0.2mM DPPH.

7.5. Determination TP and Antioxidants of Mushroom. (Valve A)

7.5.1. FIA-FCR Determination

When used valve A, the volume of extracts had pooled and adjusted to 20 mL with cold 80% methanol. (1, 2, 3, 4, 5) mL of the extract was diluted with 80% methanol to 40 mL.

7.5.2. FIA-DPPH Determination

The IC₅₀ of mushroom extract = 52.4 µg/mL DPPH, 1/IC₅₀ 19.08 mg/mL DPPH

Table 4. Determination of total phenols in mushroom using 0.2 M FCR.

Volume of MS E/M	Conc. in ppm	Peak height cm			Mean \bar{a}	SD	RSD%	TP mg/g GAE	$\bar{a} \pm t \frac{SD}{\sqrt{n}}$
		NO. of replicate (N)							
		1	2	3					
1/40	3.10	1.9	1.8	1.9	1.86	0.04	2.52	12.4	1.86 ± 0.14
2/40	5.3	2.8	2.5	2.6	2.63	0.12	4.73	10.6	2.63 ± 0.37
3/40	8.72	4.2	4.6	4.3	4.36	0.16	3.89	11.6	4.36 ± 0.51
4/40	10.0	4.6	4.7	4.8	4.7	0.08	1.73	10.0	4.7 ± 0.24
5/40	11.6	5	5.5	5.3	5.27	0.20	3.90	9.3	5.27 ± 0.62

Average TP = 10.78 ± 0.37 mg/g GAE

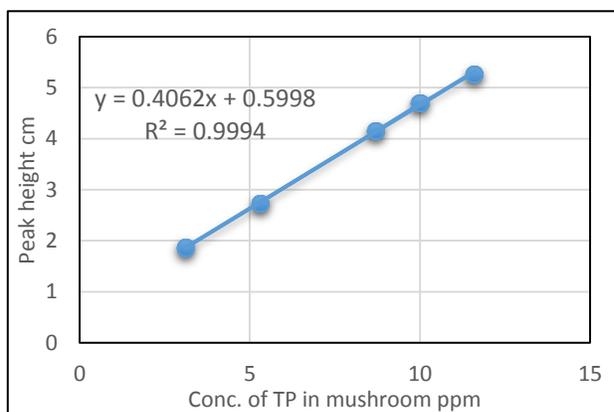


Fig. 13. The relationship between the responses expressed as an average height peak (cm), versus different concentrations of total phenols in mushroom (MS), using 0.2mM DPPH.

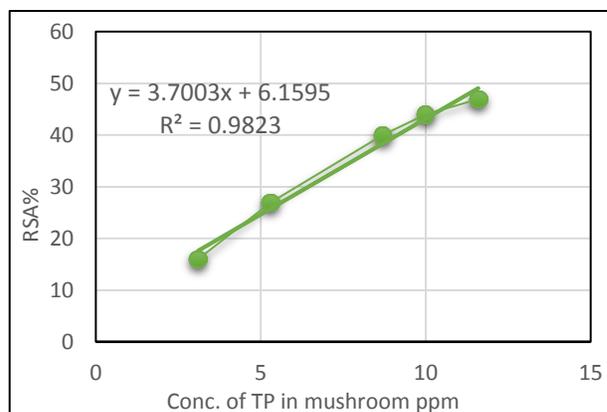


Fig. 14. The relationship between the RSA% of DPPH expressed as an average height peak (cm), versus different concentrations of mushroom (MS)

8. Results and Discussion

The developed method presented a sample consumption of 235 μL from 0.2 mM DPPH assay and 235 μL from different concentration of GA. The determination rate was 55 h^{-1} , and it was possible to achieve a determination range up to 25 ppm. The obtained limits of detection (LOD) were 0.05 ppm and 0.1 mM of GA and DPPH respectively. Different concentrations of GA were used to determine the linearity but the results showed that the calibration curve follows nonlinear relation up to 20 ppm of GA, as shown in Figs. 8, 9. The correlation coefficient r^2 was (0.9208) at 0.2mM DPPH and (0.9692) at 0.4 mM DPPH. In this study, the antioxidant activity was expressed in terms of RSA% and GA equivalent. A plot of the %RSA versus concentration of GA was prepared, and the concentration at 50% radical inhibition (IC_{50}) was determined from the linear regression equation. Practically, the IC_{50} found to be (0.105 mg/mL) and (0.137 mg/mL) when 0.2mM and 0.4mM of DPPH used respectively, the antioxidant activity of the sample was reported as IC_{50} . Hence, the lower the IC_{50} value, the higher the radical scavenging activity (RSA). The values obtained were in agreement with those described by other authors (Tibuhwa 2012).

The same standard solution was using with both valves for the determination of total phenols and antioxidant activity that are presented in Figs. 11, 13. However, the results were differed for each valve. The average values of phenolic compounds content in button mushroom, which determined by FIA-FCR, were in the range of $13.56 \pm 0.3 \text{ mg/g GAE}$ for valve B compared to $10.78 \pm 0.37 \text{ mg/g GAE}$ in valve A, The values of phenolic compounds content were approximately higher than those obtained from valve A. In addition, the determined antioxidants activity with free radical scavenging was notably higher in valve B than it in valve A. Scavenging effects of methanol extracts from mushrooms in DPPH radical increased by increasing of GA concentration. Generally, the scavenging activity determined by using valve B was higher than that of valve A of

mushroom extract in the tested concentration range. Therefore, the determination of radical scavenging activity of mushroom extract by valve B was better than that by valve A. The scavenging activity of mushroom extracts towards DPPH free radicals was expressed in terms of IC_{50} . A lower IC_{50} or a higher $1/IC_{50}$ value indicated stronger ability of the extracts to act as DPPH radical scavengers. According to the Figs 12, 14 mushroom was showed higher radical scavenging activity with IC_{50} value = 80.68 $\mu\text{g/mL}$ DPPH, $1/IC_{50}$ = 12.4 mg/mL DPPH for valve B compared to IC_{50} value = 52.4 $\mu\text{g/mL}$ DPPH, $1/IC_{50}$ = 19.08 mg/mL DPPH for valve A. The IC_{50} value for valve A was lower in comparison with that for valve B because of the consumption volume by valve A was higher than valve B. The determined values of TP and antioxidant activity in mushroom were very close to what other authors found.

9. Conclusions

Both valves A and B were successful for the determination of total phenols and antioxidant activity and they showed high sensitivity, high flexibility, extend range of linearity. As well as they were fast and easy to use. Their tools were readily available and not expensive. The measurements were indicated that the determined of total phenols and antioxidant activity which measured by the innovated valve B, were better than that measured by valve A.

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