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**ORIGINAL ARTICLE**

## **Antioxidant Activity of Methanolic and Hydroalcoholic Extracts of Garlic Plant**

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### **ABSTRACT**

The methanolic and hydroalcoholic extracts of the *Allium sativum* were investigated for its antioxidant activity and total phenolic content using high performance liquid chromatography (HPLC) analysis. The antioxidant activities were also determined by ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity. The hydroalcoholic extract had the higher flavonoid and total phenolic contents than methanolic extract of *Allium sativum*. Furthermore, a strong positive correlation of  $R^2 = 0.90$  between total phenolic content and antioxidant activity was observed in this investigation. This study indicated that *Allium sativum* exhibited the high antioxidant activity, flavonoid and phenolic contents and can be use potentially as a readily accessible source of natural antioxidant. The total phenolic content measured by the Folin-Ciocalteu procedure does not give a full picture of the quantity of the phenolic constituents in the extracts.

**Key words:** Phenolic compound; DPPH; Antioxidants; PJs; Folin-Ciocalteou; *Allium sativum*

### **Introduction**

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants [17,9,6]. Phenolics are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers [11]. Antioxidants are important because they have the ability of protecting organisms from damage caused by free radical-induced oxidative stress [2]. Besides well known and traditionally used natural antioxidants from tea, fruits, vegetables and spices, some natural antioxidant are already exploited commercially either as antioxidant additives or a nutritional supplements [14]. In this regard, although many of plant species have been investigated in the search for novel antioxidants [4], there is still a demand to find more information concerning the

antioxidant potential of plant species. Flavonoids are a group of poly phenolic compounds with known properties included free radical scavenging, inhibition of hydrolytic and oxidative enzymes, and anti-inflammatory action. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity [8,12]. According to physiological properties of different flavonoids, the flavonols having orto or para hydroxyl group in the 2- phenyl ring are known to have strong antioxidant properties, while free hydroxyl at the 5,7- position proved to have a pro-oxidant effect. Garlic (*Allium sativum*) is a remarkable plant, which has multiple beneficial effects such as antimicrobial, antithrombotic, hypolipidemic, antiarthritic, hypoglycemic and antitumor activity [16]. It is used universally as a flavoring agent, traditional medicine and a functional food to enhance physical and mental health. The beneficial effects of garlic consumption

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in treating a wide variety of human diseases and disorders have been recorded by many civilizations [13]. It has been reported to provide protection against hypercholesterolemic atherosclerosis and ischemia-reperfusion-induced arrhythmias and infarction. In the present study, the antioxidant activity, flavonoid and phenolic contents of the *Allium sativum* were investigated.

## Materials and methods

### Sample Preparation:

The samples were first dried and then ground to fine powder. 5 g of the fine powder was extracted with 300 ml of 80% methanol for 8 hours. Sample was recovered by evaporating the ethanol using Rotary Evaporator, then dissolved in 25 mL of phosphate buffer saline and stored at 4°C for further use. The supernatant was recovered and used for the DPPH assay and total phenolic analysis. HPLC analysis was used for polyphenol determination.

### Chemicals:

The DPPH and quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one) were purchased from Sigma Chemical Co. (St., Louis, USA). Gallic acid, Folin-Ciocalteu reagent, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) and methanol were purchased from Merck Co. (Germany).

### Determination of Total Phenolic Content:

The total phenolic content of the *Allium sativum* extracts was determined using the Folin-Ciocalteu reagent [18]. The reaction mixture were contained 200 µl of diluted rice bran extract, 800 µl of freshly prepared diluted Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The final mixture was diluted to 7 ml with deionized water. Mixtures were kept in dark at ambient conditions for 2 h to complete the reaction. The absorbance at 765 nm was measured. Gallic acid was used as standard and the results were expressed as mg of Gallic acid equivalents (GAE) g<sup>-1</sup> of *Allium sativum*.

### Determination of Total Flavonoid Content:

Total flavonoid content was determined using aluminium chloride (AlCl<sub>3</sub>), and quercetin (standard) as described by Ordon Ez *et al.* [10]. The plant extract of 0.1 ml was added to 0.3 ml distilled water followed by 5% NaNO<sub>2</sub> (0.03 ml). Then, AlCl<sub>3</sub> (0.03 ml, 10%) was added, after 5 min and at 25°C. After further 5 min, the reaction mixture was treated with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance

was measured at 510 nm. The results were expressed as mg quercetin (QE) g<sup>-1</sup> of *Allium sativum*.

### Antioxidant Activity:

The antioxidant activity was determined using DPPH free radical scavenging method. A dilution of 1 M DPPH was prepared and the absorbance of a mixture of 1 ml of the extract and 1 ml of the DPPH solution was measured at 517 nm. The radical scavenging activity was calculated from the following equation [1]:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

### Ferric Reducing Antioxidant Power (FRAP) Assay:

FRAP assay is based on the ability of antioxidants to reduce Fe<sup>3+</sup> to Fe<sup>2+</sup> in the presence of TPTZ, forming an intense blue Fe<sup>2+</sup>-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH of 3.6). The absorbance decrease is proportional to the antioxidant content [3], 0.2 ml of the extract is added to 3.8 ml of FRAP reagent (10 parts of 300 Mm sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20.0 mM FeCl<sub>3</sub> · 6H<sub>2</sub>O solution) and the reaction mixture is incubated at 37°C for 30 min and the increase in absorbance at 593 nm is measured. FeSO<sub>4</sub> is used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of sample is calculated from the linear calibration curve and expressed as mmol FeSO<sub>4</sub> equivalents per gram of sample. Ascorbic acid and quercetin were used as a positive control.

### Reducing Power:

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [19]. 1.0 ml extract is mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM) and incubated at 50°C for 20 min. Thereafter, 2.5 ml of trichloroacetic acid (600 mM) is added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) is mixed with 2.5 ml of distilled water and 0.5 ml of FeCl<sub>3</sub> (6 mM) and absorbance is measured at 700 nm.

### HPLC Analysis:

The analysis was performed with a flow rate of  $0.75 \text{ mL min}^{-1}$ , using 0.2% trifluoroacetic acid (TFA) as solvent A and Methanol as solvent B, with a linear gradient from 5 to 80% methanol in 50 min (Table 1). All solvent used were filtered on Acetate Plus (0.22) before analysis. The selected flavonoid standards required a greater concentration of Methanol (80%) and a longer HPLC run for their proper elution than phenolic acids. Each standard was first injected individually to determine the exact retention time and chromatographic characteristics ( $K_{\text{max}}$ , absorbance ratio) followed by the analysis of the standard mixture. The methanolic extract was filtered on qualitative circle, Whatman filter paper No. 1 (Whatman International Ltd., UK) under vacuum and subsequently on Acetate Plus filters ( $0.22 \mu\text{m}$ ) to remove the undesirable contaminants. HPLC analysis was performed using a liquid chromatographic Agilent 1200 equipped with UV detector G1314B. All the analysis was performed in triplicate and descriptive statistical analysis, Pearson correlation coefficients, one-way analysis of variance (ANOVA) were performed using SPSS.

### Results and discussion

Table 2 indicated the total phenolic and flavonoids contents and antioxidant activities of methanol and aqueous extract of *Allium sativum*. Furthermore, the retention times of some polyphenols and the HPLC chromatograms for some of polyphenol from methanolic extract were presented in Table 3 and Figure 1 respectively.

The analysis of the extract of *Allium sativum* showed scavenging effect against DPPH radical. The hierarchy for antioxidant capacity with respect to their half maximal effective concentration ( $EC_{50}$ ) values was hydroalcoholic extract > methanolic extract. Correlation coefficient showed that total phenolic content was responsible for antiradical efficiency in the extract of *Allium sativum*. The antioxidant and total phenolic content levels are also positively and significantly correlated. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable [12]. The mechanisms of action of flavonoids are through scavenging or chelating process [5,7]. Phenolic compounds are a class of antioxidant agents which act as free radical

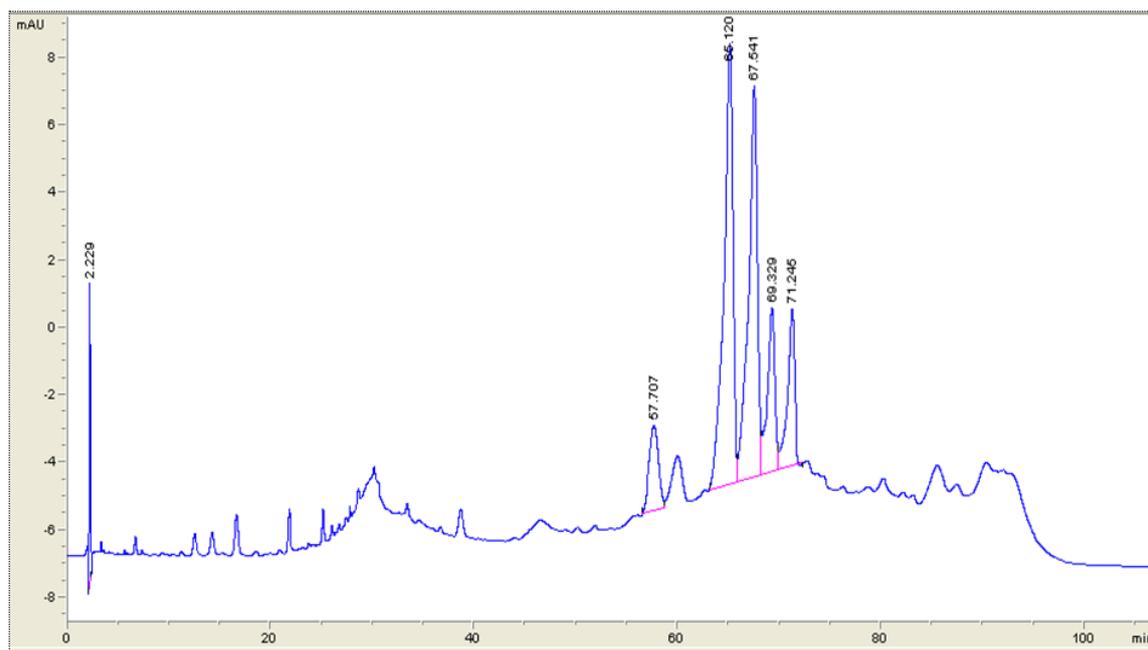
**Table 1:** Gradient elution method performed with binary solvent system using as mobile phase.

	0-15	15-25	25-40	40-70	70-80	80-90	90-100
A	95	85	85	78	78	75	95
B	5	15	78	22	22	25	5

**Table 2:** Total phenolic and flavonoids contents and antioxidant activities of methanol and hydroalcoholic extracts of *Allium sativum*.

Extracts	Phenolic content (mg GAL $\text{g}^{-1}$ )	Flavonoids content (QE $\text{g}^{-1}$ )	Antioxidant activity by DPPH (Inhibition %)	Antioxidant (FRAP) $\text{mmol g}^{-1}$
Methanolic extract	$48.2 \pm 0.0$	$5.8 \pm 0.0$	$42.0 \pm 0.1$	$38.0 \pm 0.3$
Hydroalcoholic extract	$98.2 \pm 0.0$	$7.1 \pm 0.0$	$54.9 \pm 0.1$	$46.0 \pm 0.3$

Each value in the table was obtained by calculating average of three experiments; SE ( $\pm$ ), standard error.



**Fig. 1:** HPLC chromatogram for some of polyphenol from methanolic extract

**Table 3:** Retention time of some polyphenols

Standards	Vanilic acid	Quercetin	Gallic acid	Coumaric acid	Catechin	Cafeic acid	Benzoic acid
Retention time (minute)	34.0	63.0	3.1	38.0	22.3	21.2	2.4

terminators [15]. The results suggested that the extract of *Allium sativum* can be a promising source of potential antioxidants. The antioxidant activities observed can be ascribed both to mechanisms exerted by phenolic compounds and also to synergistic effects of different phytochemicals. The total phenolic content measured by the Folin–Ciocalteu procedure does not give a full picture of the quantity of the phenolic constituents in the extracts.

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